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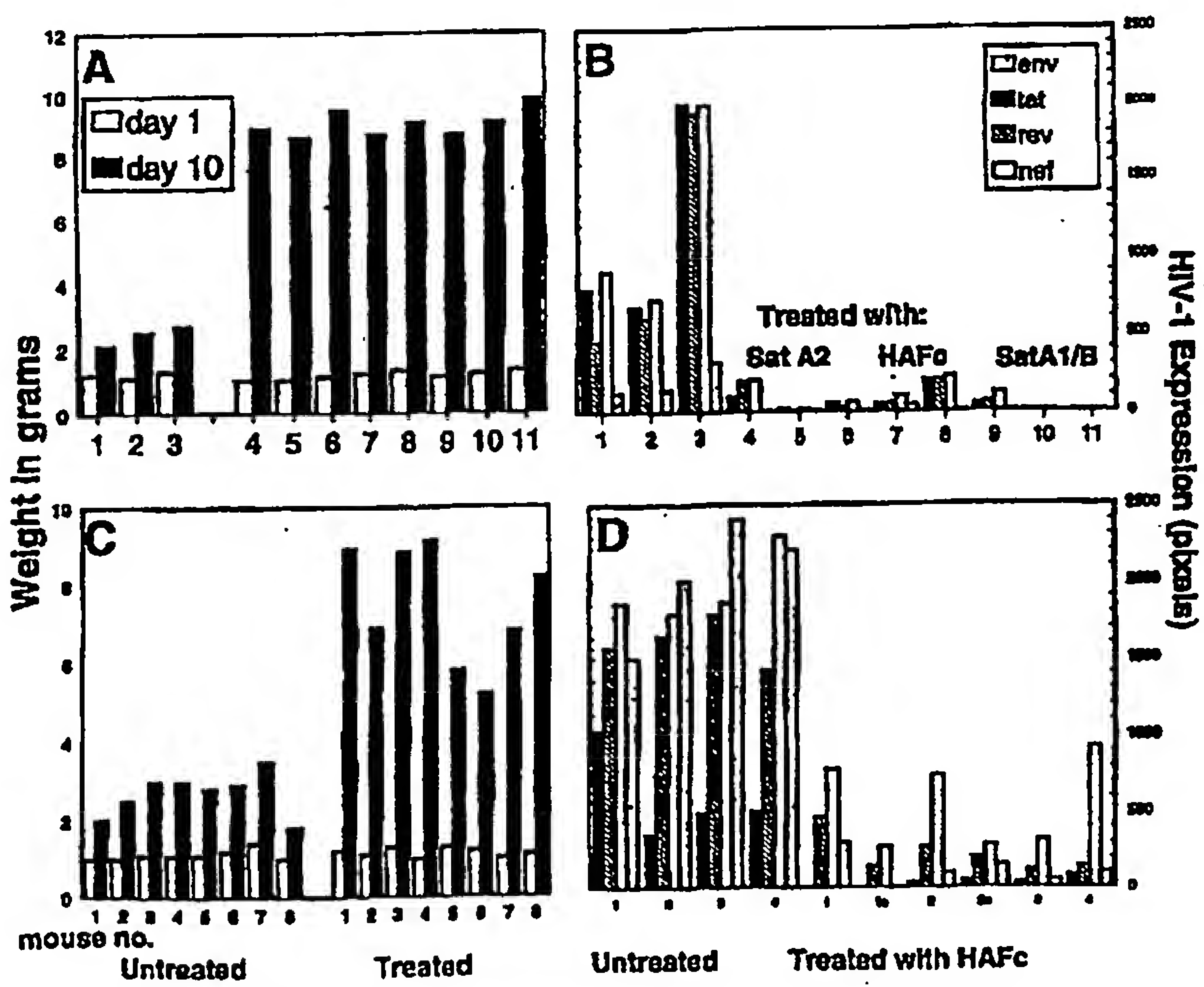
(57) Abstract

The present invention relates to β-hCG, particularly certain β-hCG peptides, and analogs and derivatives thereof. The invention also relates to fractions of a source of native hCG or native β-hCG, which fractions are active in inhibiting HIV infection or replication, against Kaposi's sarcoma or have a pro-hematopoietic effect. The invention further relates to methods of treatment and prevention of HIV infection by administration of a therapeutic compound of the invention. Such therapeutic compounds include hCG, β-hCG and β-hCG peptides, analogs and derivatives of hCG, β-hCG and β-hCG peptides, and nucleic acids encoding hCG, β-hCG and β-hCG peptides, and therapeutically and prophylactically effective fractions of sources of native hCG or native β-hCG. Pharmaceutical compositions and methods of administration of therapeutics are also provided.



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TREATMENT AND PREVENTION OF HIV INFECTION BY ADMINISTRATION OF DERIVATIVES OF HUMAN CHORIONIC GONADOTROPIN

1. CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation in part of co-pending application Serial No. 08/709,948, filed September 9, 1996 which is a continuation in part of application Serial No. 08/669,681, filed June 24, 1996, both of which are incorporated by reference herein in their entireties.

2. FIELD OF THE INVENTION

The present invention relates to peptides containing a sequence of one or more portions of the human chorionic gonadotropin β-chain as well as methods for treatment and prevention of HIV infection using human chorionic gonadotropin, the β-chain of human chorionic gonadotropin and peptides containing a sequence of one or more portions of the β-chain of human chorionic gonadotropin and derivatives thereof, for the treatment and prevention of HIV infection. The invention further relates to fractions of preparations of human chorionic gonadotropin and of human early pregnancy urine, which fractions have anti-HIV and/or anti-Kaposi's Sarcoma ("anti-KS") activity. The present invention further relates to pharmaceutical compositions for the treatment and prevention of HIV infection.

3. BACKGROUND OF THE INVENTION

3.1. THE HUMAN IMMUNODEFICIENCY VIRUS

The human immunodeficiency virus (HIV) has been implicated as the primary cause of the slowly degenerative immune system disease termed acquired immune deficiency syndrome (AIDS) (Barre-Sinoussi, F., et al., 1983, *Science* 220:868-870; Gallo, R., et al., 1984, *Science* 224:500-503). There are at least two distinct types of HIV: HIV-1 (Barre-Sinoussi, F., et al., 1983, *Science* 220:868-870; Gallo, R., et al., 1984, *Science* 224:500-503) and HIV-2 (Clavel, F., et

al., 1986, *Science* 233:343-346; Guyader, M., et al., 1987, *Nature* 326:662-669). Further, a large amount of genetic heterogeneity exists within populations of each of these types. In humans, HIV replication occurs prominently in CD4⁺ T lymphocyte populations, and HIV infection leads to depletion of this cell type and eventually to immune incompetence, opportunistic infections, neurological dysfunctions, neoplastic growth, and ultimately death.

HIV is a member of the lentivirus family of retroviruses (Teich, N., et al., 1984, *RNA Tumor Viruses*, Weiss, R., et al., eds., CSH-Press, pp. 949-956). Retroviruses are small enveloped viruses that contain a single-stranded RNA genome, and replicate via a DNA intermediate produced by a virally-encoded reverse transcriptase, an RNA-dependent DNA polymerase (Varmus, H., 1988, *Science* 240:1427-1439).

The HIV viral particle comprises a viral core, composed in part of capsid proteins, together with the viral RNA genome and those enzymes required for early replicative events. Myristylated gag protein forms an outer shell around the viral core, which is, in turn, surrounded by a lipid membrane envelope derived from the infected cell membrane. The HIV envelope surface glycoproteins are synthesized as a single 160 kilodalton precursor protein which is cleaved by a cellular protease during viral budding into two glycoproteins, gp41 and gp120. gp41 is a transmembrane glycoprotein and gp120 is an extracellular glycoprotein which remains non-covalently associated with gp41, possibly in a trimeric or multimeric form (Hammarskjold, M., & Rekosh, D., 1989, *Biochem. Biophys. Acta* 989:269-280).

HIV is targeted to CD4⁺ cells because a CD4 cell surface protein (CD4) acts as the cellular receptor for the HIV-1 virus (Dalgleish, A., et al., 1984, *Nature* 312:763-767; Klatzmann et al., 1984, *Nature* 312:767-768; Maddon et al., 1986, *Cell* 47:333-348). Viral entry into cells is dependent upon gp120 binding the cellular CD4 receptor molecules (McDougal, J.S., et al., 1986, *Science* 231:382-385; Maddon, P.J., et al., 1986, *Cell* 47:333-348), explaining HIV's

tropism for CD4+ cells, while gp41 anchors the envelope glycoprotein complex in the viral membrane. While these virus:cell interactions are necessary for infection, there is evidence that additional virus:cell interactions are also required.

3.2. HIV TREATMENT

HIV infection is pandemic and HIV-associated diseases represent a major world health problem. Although considerable effort is being put into the design of effective Therapeutics, currently no curative anti-retroviral drugs against AIDS exist. In attempts to develop such drugs, several stages of the HIV life cycle have been considered as targets for therapeutic intervention (Mitsuya, H., et al., 1991, *FASEB J.* 5:2369-2381). Many viral targets for intervention with HIV life cycle have been suggested, as the prevailing view is that interference with a host cell protein would have deleterious side effects. For example, virally encoded reverse transcriptase has been one focus of drug development. A number of reverse-transcriptase-targeted drugs, including 2',3'-dideoxynucleoside analogs such as AZT, ddI, ddC, and d4T have been developed which have been shown to been active against HIV (Mitsuya, H., et al., 1991, *Science* 249:1533-1544).

The new treatment regimens for HIV-1 show that a combination of anti-HIV compounds, which target reverse transcriptase (RT), such as azidothymidine (AZT), lamivudine (3TC), dideoxyinosine (ddI), dideoxycytidine (ddC) used in combination with an HIV-1 protease inhibitor have a far greater effect (2 to 3 logs reduction) on viral load compared to AZT alone (about 1 log reduction). For example, impressive results have recently been obtained with a combination of AZT, ddI, 3TC and ritonavir (Perelson, A.S., et al., 1996, *Science* 15:1582-1586). However, it is likely that long-term use of combinations of these chemicals will lead to toxicity, especially to the bone marrow. Long-term cytotoxic therapy may also lead to suppression of CD8+ T

cells, which are essential to the control of HIV, via killer cell activity (Blazevic, V., et al., 1995, *AIDS Res. Hum. Retroviruses* 11:1335-1342) and by the release of suppressive factors, notably the chemokines Rantes, MIP-1α and MIP-1β (Cocchi, F., et al., 1995, *Science* 270:1811-1815). Another major concern in long-term chemical anti-retroviral therapy is the development of HIV mutations with partial or complete resistance (Lange, J.M., 1995, *AIDS Res. Hum. Retroviruses* 10:S77-82). It is thought that such mutations may be an inevitable consequence of anti-viral therapy. The pattern of disappearance of wild-type virus and appearance of mutant virus due to treatment, combined with coincidental decline in $CD4^+$ T cell numbers strongly suggests that, at least with some compounds, the appearance of viral mutants is a major underlying factor in the failure of AIDS therapy.

Attempts are also being made to develop drugs which can inhibit viral entry into the cell, the earliest stage of HIV infection. Here, the focus has thus far been on CD4, the cell surface receptor for HIV. Recombinant soluble CD4, for example, has been shown to inhibit infection of $CD4^+$ T cells by some HIV-1 strains (Smith, D.H., et al., 1987, *Science* 238:1704-1707). Certain primary HIV-1 isolates, however, are relatively less sensitive to inhibition by recombinant CD4 (Daar, E., et al., 1990, *Proc. Natl. Acad. Sci. USA* 87:6574-6579). In addition, recombinant soluble CD4 clinical trials have produced inconclusive results (Schooley, R., et al., 1990, *Ann. Int. Med.* 112:247-253; Kahn, J.O., et al., 1990, *Ann. Int. Med.* 112:254-261; Yarchoan, R., et al., 1989, Proc. Vth Int. Conf. on AIDS, p. 564, MCP 137).

The late stages of HIV replication, which involve crucial virus-specific processing of certain viral encoded proteins, have also been suggested as possible anti-HIV drug targets. Late stage processing is dependent on the activity of a viral protease, and drugs are being developed which inhibit this protease (Erickson, J., 1990, *Science* 249:527-533).

Recently, chemokines produced by $CD8^+$ T cells have been implicated in suppression of HIV infection (Paul, W.E., 1994, *Cell* 82:177; Bolognesi, D.P., 1993, *Semin. Immunol.* 5:203). The chemokines RANTES, MIP-1α and MIP-1β, which are secreted by $CD8^+$ T cells, were shown to suppress HIV-1 p24 antigen production in cells infected with HIV-1 or HIV-2 isolates *in vitro* (Cocchi, F, et al., 1995, *Science* 270:1811-1815). Thus, these and other chemokines may prove useful in therapies for HIV infection. The clinical outcome, however, of all these and other candidate drugs is still in question.

Attention is also being given to the development of vaccines for the treatment of HIV infection. The HIV-1 envelope proteins (gp160, gp120, gp41) have been shown to be the major antigens for anti-HIV antibodies present in AIDS patients (Barin et al., 1985, *Science* 228:1094-1096). Thus far, therefore, these proteins seem to be the most promising candidates to act as antigens for anti-HIV vaccine development. Several groups have begun to use various portions of gp160, gp120, and/or gp41 as immunogenic targets for the host immune system. See for example, Ivanoff, L., et al., U.S. Pat. No. 5,141,867; Saith, G., et al., WO92/22,654; Shafferman, A., WO91/09,872; Formoso, C., et al., WO90/07,119. To this end, vaccines directed against HIV proteins are problematic in that the virus mutates rapidly rendering many of these vaccines ineffective. Clinical results concerning these candidate vaccines, however, still remain far in the future.

Thus, although a great deal of effort is being directed to the design and testing of anti-retroviral drugs, effective, non-toxic treatments are still needed.

3.3. HUMAN CHORIONIC GONADOTROPIN

Human chorionic gonadotropin (hCG), which is required for the maintenance of pregnancy, is a member of the glycoprotein hormone family. The glycoprotein hormones, which also include follicle-stimulating hormone (FSH), luteinizing hormone (LH) and thyroid-stimulating hormone

(TSH), consist of two sub-units, α and β. These subunits are non-covalently linked to form a heterodimer, and heterodimer formation has been shown to b required for receptor binding. Within a particular species, the α-subunits are identical among the glycoprotein hormones while the β-subunits differ and determine the receptor binding specificity of the particular hormone (Kornyei, J.L., et al., 1993, *Biol. Reprod.* 49:1149). The β-subunits of the glycoprotein hormones exhibit a high degree of sequence similarity within the N-terminal 114 amino acids. LH is the most similar to hCG with 85% sequence homology within the first 114 amino acids, and both proteins bind the same receptor. hCG, however, contains a C-terminal extension not present in the other glycoprotein β-chains (Lapthorn, A.J., et al., 1994, *Science* 369:455-461).

From the three dimensional crystal structure of hCG, it was determined that hCG, like the growth factors nerve growth factor (NGF), transforming growth factor-β (TGF-β) and platelet-derived growth factor-β (PDGF-β), is a cysteine-knot glycoprotein. Proteins containing such a cysteine-knot motif have at least three disulfide bridges, two of which join adjacent anti-parallel strands of the peptide, thus, forming a ring, and one of which joins the peptide chain through the ring. Particular structures in the hCG β-chain include the determinant loop sequence (β93-100) which has been implicated in subunit association and the longest inter-cysteine loop (β38-57) which may play a role in receptor binding. Residues 47-53 appear to be exposed at the surface of this inter-cysteine loop (Lapthorn et al., 1994, *Nature* 369:455-461).

Previously, purified preparations of heterodimeric hCG have been shown to reduce the reverse transcriptase activity in HIV-1 infected lymphocytes and monocytes in culture (Bourinbaiar, A.S., and Nagorny, R., 1992, *FEMS Microbiology Letters* 96:27-30) and to prevent transmission of HIV from lymphocytes to trophoblasts in vitro (Bourinbaiar, A.S., and Nagorny, R., 1992, *FEBS Letters* 309:82-84). Additionally, the β-subunit of hCG (β-hCG) has been demonstrated to reduce

HIV production in lymphocytes at doses from 100 pg/ml to 100 μg/ml and in monocytes at doses up to approximately 10 μg/ml, with higher doses actually increasing the level of viral production in monocytes (Bourinbaiar, A.S., and Lee-Huang, S., 1995, *Immunology Letters* 44:13-17). However, none of these reports disclose the potential efficacy of β-hCG peptides in HIV inhibition *in vitro* or of hCG or any portion or derivative thereof in HIV treatment or prevention *in vivo*.

Furthermore, doses of hCG below those necessary to induce a humoral immune response have been proposed for treatment of HIV infection based on observations of therapeutic effects of such doses on cats and cows infected with feline leukemia and bovine leukemia viruses respectively (U.S. Patent No. 4,880,626). This patent suggested use of the hCG dimer at very low doses (approximately 2 I.U. per treatment).

Lunardi-Iskandar et al. (1995, *Nature* 375:64-68 and PCT Application WO96/04008) reported that hCG, β-hCG, as well as a β-hCG carboxy-terminal peptides of amino acids 109-145 (SEQ ID NO:25) and 109-119 (SEQ ID NO:7) are efficacious in the treatment of Kaposi's Sarcoma. However, neither reference discloses or even suggests that hCG, β-hCG or β-hCG peptides of amino acids 109-145 or 109-119 (SEQ ID NOS:7 and 25, respectively) have any viral anti-activity or that other β-hCG peptides have any therapeutic activity.

Finally, Harris (1995, *The Lancet* 346:118-119) reported that treatment with hCG improved T cell counts and physical symptoms in certain HIV infected subjects.

Citation of references hereinabove shall not be construed as an admission that such references are prior art to the present invention.

4. SUMMARY OF THE INVENTION

The present inventors have found that hCG preparations, β-hCG preparations, certain peptides of β-hCG, certain combinations of β-hCG peptides linked via their N-termini and C-termini by peptid bond(s), and certain gel filtration

chromatography fractions of commercial hCG preparations and human early (*i.e.* first trimester) pregnancy urine exhibit anti-viral activities. In particular, hCG and β-hCG preparations and certain gel filtration chromatography fractions of commercial hCG preparations and of human early pregnancy urine, as described by way of example hereinbelow, and specific peptides thereof inhibit HIV-1 replication *in vitro*, inhibit HIV-1 gene-expression in HIV-1 transgenic mice, reduce plasma virus levels in SIV infected monkeys and in AIDS patients, and increase $CD4^+$ T cells in HIV transgenic mice, SIV infected monkeys and AIDS patients. The present inventors have further found that the subjects tolerated treatment with hCG and β-hCG preparations very well and that the virus did not become resistant to treatment after exposure to hCG or β-hCG. The present invention fills a tremendous need for a non-toxic, long-term treatment of HIV infection and its sequelae, ARC and AIDS.

The present invention relates to proteins having a sequence of one or more portions of the β-chain of hCG (β-hCG), particularly proteins having the sequence of amino acid numbers 41-54, 45-54, 47-53, 45-57 and 109-119 (SEQ ID NOS:3-7, respectively). The present invention also relates to proteins comprising or, alternatively, consisting of, the sequence of two or more portions of β-hCG, *e.g.*, wherein said portions are linked via their N-termini and C-termini by peptide bond(s), particularly proteins having the sequence of amino acid numbers 45-57 (SEQ ID NO:6) linked via a peptide bond at the C-terminus to the N-terminus of a peptide of amino acid numbers 109-119 (SEQ ID NO:7) or linked at the N-terminus to the C-terminus of a peptide of amino acid numbers 110-119 (SEQ ID NO:27); or a peptide of amino acid numbers 47-57 (SEQ ID NO:28) linked by a peptide bond at the C-terminus to the N-terminus of a peptide of amino acid numbers 108-119 (SEQ ID NO:29) of β-hCG as depicted in Figure 8 (a portion of SEQ ID NO:2), *i.e.* the peptides denoted 45-57::109-119, 110-119::45-57, or 47-57::108-119 (SEQ ID NOS:30-32, respectively). The present invention also relates

to certain fractions (*i.e.* components of a source of hCG or β-hCG isolated away from other components in the source of hCG or β-hCG by a separation technique known in the art) of any source of hCG or β-hCG, such as commercial hCG preparations and human (preferably early, *i.e.*, first trimester) pregnancy urine, which fractions have anti-HIV and/or anti-Kaposi's Sarcoma activity.

The present invention further relates to therapeutic methods and compositions for treatment and prevention of diseases and disorders associated with HIV infection based on hCG and β-hCG preparations, therapeutically and prophylactically effective fractions of a source of hCG or β-hCG (preferably a source of native hCG or β-hCG, *i.e.* a source of naturally occurring hCG or β-hCG, not recombinantly produced hCG or β-hCG) and therapeutically and prophylactically effective proteins containing a sequence of one or more portions (*i.e.*, a fusion protein comprising more than one β-hCG peptide sequence either as non-contiguous or contiguous sequences, *e.g.*, having an amino acid sequence of one β-hCG peptide linked via a peptide bond to another β-hCG peptide) of β-hCG, and related derivatives and analogs. The invention provides for treatment and prevention of HIV infection by administration of a therapeutic compound of the invention. The therapeutic compounds of the invention include: hCG, β-hCG, therapeutically and prophylactically effective fractions of a source of hCG or β-hCG, therapeutically and prophylactically effective peptides having a sequence of a one or more portions of β-hCG, modified derivatives of hCG, β-hCG and β-hCG peptides, and nucleic acids encoding β-hCG and therapeutically and prophylactically effective peptides having a sequence of one or more portions of β-hCG, and derivatives and analogs of the foregoing. The invention also provides *in vitro* and *in vivo* assays for assessing the efficacy of therapeutics of the invention for treatment or prevention of HIV. The invention also provides pharmaceutical compositions and methods of

administration of therapeutics of the inv ntion for treatment or prevention of HIV infection.

4.1. DEFINITIONS

As used herein, the following terms shall have the meaning indicated.

AIDS	Acquired Immune Deficiency Syndrome
ARC	AIDS-Related Complex
hCG	Human Chorionic Gonadotropin
KS	Kaposi's Sarcoma
OI	Opportunistic Infection
PBMC	Peripheral Blood Mononuclear Cell

5. DESCRIPTION OF THE FIGURES

Figures 1A-E. Effects of an hCG preparation, APL™ (Wyeth-Ayerst), β-hCG peptides and certain fractions of hCG APL™ and early preganancy urine on weight and HIV-1 gene expression in HIV-1 transgenic mice. (A) and (C) Weight change in grams in individual HIV-1 transgenic mice after treatment from from day 1 to day 10 post partum is represented as a bar graph with open bars representing the weight at day 1 and solid bars representing the weight at day 10. (B), (D) and (E) Suppression of HIV-1 gene expression in transgenic mice. The bar graph presents the level of expression in pixels, as determined by chemiluminescence assay of the HIV genes <u>env</u>, <u>tat</u>, <u>rev</u>, and <u>nef</u> in the individual HIV transgenic mice. For (B) and (D), the black bars represent <u>tat</u> expression, the striped bars represent <u>rev</u> expression, the lightly stippled bars represent <u>env</u> expression, the open bars represent <u>nef</u> expression. For (E), the striped bars represent <u>env</u> expression, the solid bars represent <u>rev</u> expression, and the open bars represent <u>nef</u> expression. In (A) and (B), bars 1-3 represent untreated control transgenic mice; bars 4-6 represent mice whose mothers received (subjects were administered through the mothers' milk) 200 μg circularized β-hCG peptide 44-57 (with cysteine substituted at position 44; SEQ ID NO:26) per day;

bars 7-9 represent mice whose mothers received 300 IU per day hCG-APL™; and bars 9-11 represent mice whose mothers received 200 μg per day of the fus d β-hCG peptide 45-57::109-119 (SEQ ID NO:30). In (C) and (D), the bars labeled "untreated" represent mice receiving only PBS; and the bars labeled "treated" or "treated with HAFc" represent mice administered 300 IU per day of hCG APL™. In (E), the set of bars labeled "1" represents mice treated with PBS alone; "2" represents mice treated with 100 μg β-hCG core peptide per day; "3" represents mice treated with 100 μg per day α-hCG; "4" represents mice treated with 200 μl per day of fraction 61 of the hCG APL™ fractionation; and "5" represents mice treated with 200 μl per day of fraction 65 of the early pregnancy urine fractionation.

Figures 2A-D. Effects of an hCG preparation on indicators of SIV infection in SIV-infected macaques. SIV was given intravenously at a dose of $10^{4.5}$ $TCID_{50}$ per ml. (A) SIV titer was monitored over time in months by quantifying the p27 gag protein (Organon Teknika assay) as nanograms (ng) of p27/ml of plasma from the plasma of the SIV infected macaques. Treated SIV-infected macaques (indicated as Rx) were given hCG APL™, 3000 IU, 2x weekly. Plasma levels of p27 gag in these treated monkeys are indicated on the graph by lines with diamonds, number (#) signs or filled circles. Results with the untreated SIV-infected macaques (indicated UnRx) are indicated by the lines with either stars or triangles. (B) $CD4^+$ T cell levels were determined in cells/mm^3 in SIV-infected macaques either treated with hCG or untreated over time in months. Results from the SIV-infected monkeys treated with hCG (APL™) (Rx) are indicated by lines with diamonds, number (#) signs or filled circles, while results with the untreated monkeys (UnRx) are indicated by lines with stars or triangles. (C) Change in weight in kilograms (kg) was monitored in treated and untreated SIV-infected monkeys over time in months. Weight changes in the SIV-infected monkeys treated with hCG (APL™) (Rx) are indicated by lines with diamonds, # signs or filled circles,

while results in the untreated monkeys (UnRx) are indicated by lines with stars or triangles. (D) Levels of $CD4^+$ T cells were monitored in normal uninfected monkeys either treated with hCG (APL™) or untreated over time in months. $CD4^+$ T cell levels in the untreated monkeys are indicated by lines with sun-like figures or squares, and the results in the treated monkeys are indicated by lines with pentagonal figures or with filled inverted triangles.

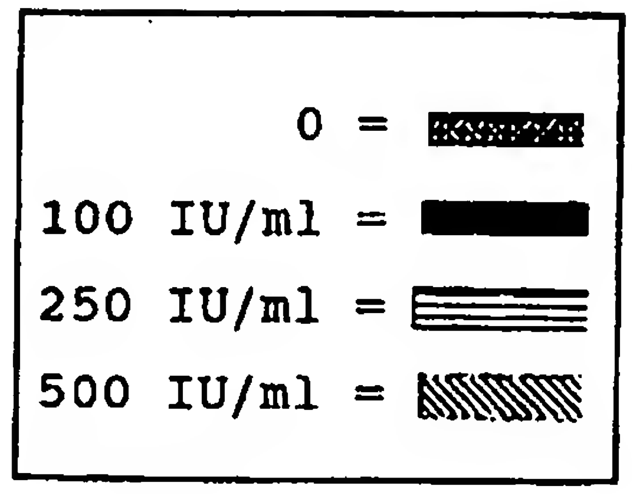
Figures 3A-J. Effects of administration of hCG preparations on HIV-1 viral load and $CD4^+$ T cell levels in individual patients in the clinical study described in Section 7.3 *infra*. Figures A and B are data from patient PHOJ, C and D from patient PG1, E and F from patient PG3, G and H from patient PHVE, and I and J from patient PG17. In panels A, C, E, G and I, viral load and $CD4^+$ T Cell counts are plotted over time (in months). Viral load (measured by RT-PCR in panels A and G and by the Roche Amplicor test in panels C, E and I) is plotted as the logarithm of the viral load (represented by line with "X" data points). The $CD4^+$ T Cell levels are plotted as $CD4^+$ T Cells/ml (represented by line with triangle data points). Panels B, D, F, H, and J plot the dosage of hCG in IU (X 1000) per week over time in months, with the timing of other therapies indicated above the graph with a thick arrow.

Figures 4A-D. Effects of hCG preparations and peptides on HIV replication *in vitro*. (A and B) These graphs depict the concentration dependence of inhibition of HIV-1 IIIB infection of $CD4^+$ T cells or total PBMCs from peripheral blood of normal donors (infection is expressed in nanograms (ng) of p24/ml plasma) as a function of nmol per ml α-hCG subunit, β-hCG peptide or α-hCG peptide over a concentration of 0.05 to 50 nmol/ml. Graphs present data on infection of (A) $CD4^+$ T cells infected by HIV-1 IIIB and (B) PBMCs infected with HIV-1 IIIB. In both graphs, results with α-hCG subunit are represented by lines with diamonds, results with the β-hCG peptide 6-16 by lines with squares, results with the β-hCG peptide 109-119 by lines with stars, results with the

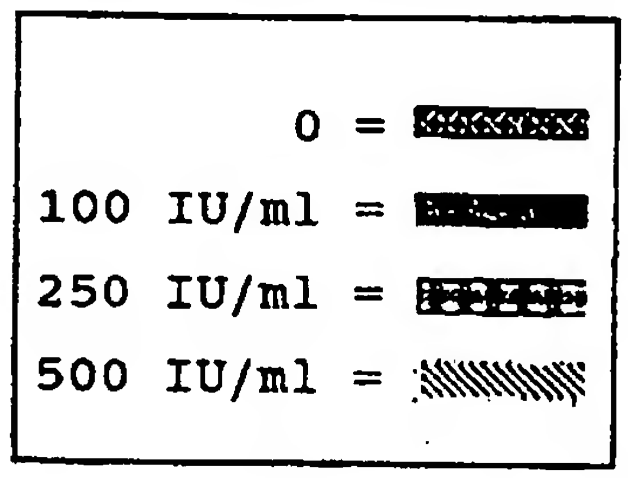
circularized β-hCG peptide 44-57 (with cysteine substituted for the amino acid at position 44) (SEQ ID NO:26) by lines with triangles, and results with the α-hCG peptide 88-92 by lines with inverted triangles. (C and D) These graphs depict data on the effect of α-hCG, hCG peptides and commercial hCG preparations on the infection of primary macrophages by HIV-1 Ba-L. (C) Effect of different concentrations of hCG α subunits and various hCG peptides (0.05 to 50 nmol/ml) on infection in peripheral blood macrophages from a normal donor infected with HIV-1 Ba-L. The results are averages of triplicate samples with less than 15% variation. Results with α-hCG subunit are represented by lines with diamonds, results with the β-hCG peptide 6-16 by lines with squares, results with the β-hCG peptide 109-119 (SEQ ID NO:7) by lines with stars, results with the circularized β-hCG peptide 44-57 (with cysteine substituted for the amino acid at position 44) (SEQ ID NO:26) by lines with triangles, and results with the α-hCG peptide 88-92 by lines with inverted triangles. (D) A comparison of the inhibition of HIV Ba-L infection of macrophages by different commercial native hCG preparations (APL™, Wyeth Ayerst; STERIS™, Steris; PREGNYL™, Organon) and by purified hCG (CR127) over a concentration range of 0.05 IU to 1,000 IU/ml. Results are shown at day 10. Results with APL™ hCG are indicated by a line with inverted triangles, results with STERIS™ hCG are indicated by a line with circles, results with PREGNYL™ hCG are indicated by a line with squares, and results with hCG-CR127 are indicated by a line with X's.

Figures 5A-C. Effect of preparations of hCG on HIV-LTR activity. (A) The CAT activity of cells containing the HIV-LTR construct and treated with hCG concentrations of 0 IU/ml, 100 IU/ml, 250 IU/ml and 500 IU/ml was calculated relative to the untreated control. (B) Relative CAT activity of the unrelated SV40 promoter in response to hCG at 0 IU/ml, 250 IU/ml and 500 IU/ml was similarly calculated. Data in both A and B represents the mean +/- S.E.M. of 3 to 7 indep ndent experiments and is presented as a bar graph. The different

hCG concentrations are indicated in both A and B as depicted below:



(C) The viability of Hut 78 cells in the presence of hCG at 0 IU/ml, 250 IU/ml and 500 IU/ml under the conditions used in the transient expression assays shown in Figures A and B was determined using a coulter cell counter. Results are presented as a bar graph in terms of cell number x 10,000 and the hCG concentrations are indicated as shown below:



Figures 6A-H. Effect of hCG preparations and peptides on KS colony growth *in vitro* and KS tumors *in vivo*. (A) Comparison of the anti-KS *in vitro* effects (tumor cell killing) of purified hCG and β-hCG peptides in KS clonogenic assays using KS Y-12 and KS "SKL"18 cells depicted in a bar graph in terms of number of colonies. The results are averages of 3 sets of results with less than 10% variation and are representative of multiple experiments. Results with no hCG or hCG peptides are represented by open bars, the results with the β-hCG peptide of amino acids 109-119 (SEQ ID NO:7) are represented by stippled bars, the results with the β-hCG peptide of amino acids 109-145 (SEQ ID NO:25) are represented by the bars with horizontal stripes, the results with the circular β-hCG peptide of amino acids 44-57 (SEQ ID NO:26) where the amino acid at position 44 is a cysteine are

represented by the bars with diagonal stripes, and the results with the highly purified hCG preparation, CR 127, are represented by solid bars. (B-H) Thin sections of KS tumors induced in nude mice by inoculation with 10^6 neoplastic KS Y-1 cells. (B) Thin section of tumors from mice that were not treated with hCG or hCG subunits or peptides (C) Thin section of a tumor from a mouse after treatment with crude hCG APL™ (100 IU) subcutaneously daily for 7 days. (D) Thin section of a tumor from a mouse treated with the β-hCG peptide of amino acids 45-57 (SEQ ID NO:6), 10 μg/ml/daily (6.7 nmoles) for 5 days. (E) Thin section of a tumor from a mouse after 5 days of treatment with the circularized β-hCG peptide 44-57 where cysteine has been substituted at position 44 (SEQ ID NO:26), at 10 μg per day. (F) This panel shows the thin tissue section of KS tumor from AIDS-KS patients treated with 1 ml of diluent alone shows less than 2% cell death as detected by specific apoptosis in situ immunostaining. (G) Thin tissue section of KS tumor from an AIDS-KS patient after hCG preparation therapy of intralesional injections of 2000 IU, 3 times weekly for 2-3 weeks, shows evidence of apoptosis in all cells. (H) Thin tissue section of KS tumor from an AIDS-KS patient after hCG preparation therapy, 500 IU, 3 times weekly for 3 weeks.

Figures 7A-C. These bar graphs demonstrate the effects of hCG preparations and peptides on hematopoiesis *in vitro*. (A) This bar graph depicts results of colony assays in terms of number of colonies for CFU-MIX (colony forming units of megakaryocytes, erythrocytes, granulocytes and monocytes). (B) This bar graph presents data from colony assays for BFU-e (Burst forming units of erythrocytes) in terms of number of colonies. (C) This bar graph presents results from colony assays of CFU-GM (colony forming units of granulo-macrophages) in terms of number of colonies. For all three graphs, results are shown for cells isolated from cord blood ("cord") and bone marrow ("marrow"). The results are averages of 3 sets of results with less than 10% variation and are representative of multiple experiments. The results

from no treatment are indicated by open bars; the results with α-hCG are represented by solid bars; the results with APL™ hCG (hCGapl) are represented by bars with a lattice pattern; the results with native β-hCG preparation (NbhCG) are represented by cross-hatched bars; the results with the highly purified hCG preparation (CR127) are represented by open bars; the results with the β-hCG peptide of amino acids 109-119 (SEQ ID NO:7) (b109-119) are shown by the diagonally stippled bars; the results with the β-hCG peptide of amino acids 45-57 (SEQ ID NO:6) (b45-57) are shown by the bars with the diamonds; the results with the circularized β-hCG peptide of amino acids 44-57 with cysteine substituted for the amino acid at position 44 (SEQ ID NO:26) (b45-57c) are represented by the diagonally striped bars; and the results with the mixture of scrambled β-hCG peptides of amino acids 45-57 and 109-119 (bmix45+109) are represented by the vertically striped bars.

Figure 8. Nucleotide and amino acid sequences of β-hCG (SEQ ID NOS:1 and 2).

Figures 9A and B. Schematic depiction of the structures of (A) the linear peptide of amino acids 45-57 (SEQ ID NO:6) of the β-hCG sequence depicted in Figure 8 (SEQ ID NO:2) where the amino acid residues at positions 47 and 51 are substituted by a branch made up of diaminobutyric acid peptide bonded to proline, and (B) the circularized peptide of amino acids 44-57 (SEQ ID NO:26) with valine at position 44 substituted with cysteine, which protein is circularized via a disulfide bond between its amino- and carboxy-terminal cysteines. In both A and B, amino acids are represented by their three letter amino acid code, except for the branched residues and the terminal cysteines, for which the structure is depicted.

Figures 10A-F. These graphs depict results from the fractionation by Superdex 200 gel filtration of a commercial hCG preparation APL™ (Wyeth Ayerst) and early pregnancy urine. (A) and (D). These graphs depicts the relative amount of protein in mg/ml in each fraction identifi d by fraction

number in the hCG APL™ fractionation (A) and early pregnancy urine fractionation (D). The fractions containing the hCG dimer and β-core protein are identified with arrows and the labels "hCG" and "β-core" respectively. (B) and (E). These graphs present the percent inhibition of growth of cultured KS cells by the individual fractions from the hCG APL™ (B) and early pregnancy urine (E) using KS cell clonogenic assays. The results are plotted as percent inhibition versus fraction number. (C) and (F). These graphs depict the effect of the hCG APL™ (C) and early pregnancy urine (F) fractions on HIV replication in vitro. Specifically, this graph presents data on the percentage inhibition of HIV-1 IIIB viral infection of PBMCs as a function of fraction number.

Figure 11. Effect of hCG preparations, hCG and early pregnancy urine fractions, and β-hCG peptides on KS cell growth in vitro. Results for clonogenic assays using the cell lines KS Y-1 (bars labelled "KS Y-1") and KS SLK (labelled "KS SLK") are presented on a bar graph as percent inhibition of KS Colony Growth. Bars 1-4 represent cells treated with 200 IU/ml of the commercial hCG preparations hCG APL™, hCG CG10 (Sigma), hCG PROFASI™, and hCG PREGNYL™, respectively; bar 5 represents treatment with 50 μg/ml β-hCG core protein; bar 6, 50 μg/ml native β-hCG; bar 7, 50μg/ml native α-hCG; bar 8, 200 IU/ml highly purified hCG preparation CR 127; bar 9, 50 μg/ml recombinantly produced hCG (Sigma); bars 10 and 11, 50-100 μl/ml of fractions 65 and 76, respectively, of the early pregnancy urine fractionation; bars 12 and 13, 50-100 μl/ml of fractions 65 and 76, respectively, of the hCG APL™ fractionation; and bar 14, 100 μg/ml of the circularized β-hCG peptide 44-57 (with cysteine substituted at postion 44; SEQ ID NO:26).

Figures 12A-C. These bar graphs demonstrate the effects of hCG preparations, fractions and peptides on hematopoiesis *in vitro*. (A) Results of colony assays in terms of percent increase of hematopoiesis for CFU-GEMM (colony forming units of megakaryocytes, erythrocytes, granulocytes and monocytes). (B) Data from colony assays for BFU-e (Burst forming units of

erythrocytes) in terms of percent increase of hematopoiesis. (C) R sults from colony assays of CFU-GM (colony forming units of granulo-macrophages) in terms of percent increase of hematopoiesis. In all three graphs, bar 1 represents results from treatment with PBS alone; bar 2, the results with 100 μg/ml α-hCG; bar 3, with 200 IU/ml APL™ hCG; bar 4, 200 IU/ml of the highly purified hCG preparation CR 127; bar 5, 100 μg/ml native β-hCG preparation; bar 6, 100 μg/ml of the circularized β-hCG peptide of amino acids 44-57 with cysteine substituted for the amino acid at position 44 (SEQ ID NO:26); bar 7, 100 μl/ml of fraction 65 of the hCG APL™ fractionation; bars 8 and 9, 100 μl/ml of fractions 65 and 26, respectively, of the early pregnancy urine fractionation; and bar 10, 100 μg/ml of the β-hCG core protein.

Figure 13. Effect of treatment with hCG commercial preparation and early pregnancy urine fractions on KS tumors in mice. Results are plotted as percent inhibition of tumor size as compared to control tumors. The open bar represents mice treated with PBS alone; the solid bar with 100 IU per day of hCG APL™; diagonally striped bars with 200 μl per day of fractions 26, 76, 65, and 82 (as labelled on top of the bars) of the early pregnancy urine fractionation ("HAF-UF#"); and open bars with 200 μl per day of fractions 62, 65, 74, 76 and 35 (as labelled on top of the bars) of the hCG APL™ fractionation ("HAF-CF#").

Figure 14. Effect of hCG preparations, peptides and fractions on survival of HIV-1 transgenic mice. Data is plotted on a bar graph as "% Survival of Tg26 mice" 10 days after birth, each bar representing 4 mice. None of the untreated mice survived. Bar labelled "ahCG" represents mice treated with 200 μg per day native α-hCG; bar labelled "CR127", with 300 IU per day of the highly purified hCG preparation CR 127; bar 1, 200 μg per day native β-hCG; bar 2, 300 IU per day hCG APL™; bar labelled "rβhCG", with 200 μg per day recombinant β-hCG; bar 3, with 50 μg per day LH (l utinizing hormone); bar 4, with 200 μl per day fraction 65 of the hCG APL™ fractionation; bars 5 and 6, 200 μl p r day

fractions 65 and 76, respectively, of the early pregnancy urine fractionation; bars labell d "HAF-UF#26 and "HAF-CF#26", 200 μl per day fraction 26 from the early pregnancy urine and hCG APL™ fractionations, respectively; bar labelled "b-core", 50 μg per day β-hCG core peptide; and bar 7, with 300 μg per day circularized β-hCG peptide 44-57 (with cysteine substituted at position 44; SEQ ID NO:26).

Figures 15A-C. Graphs of change in viral load and $CD4^+$ T cell levels with hCG therapy. (A) The change in viral load is plotted as the logarithm of viral load after therapy ("Logload") as a function of viral load before therapy ("Baselog"). (B) The change in $CD4^+$ T cell levels is plotted as $CD4^+$ T cell levels after therapy (in $CD4^+$ T cells/ml) ("CD4") as a function of $CD4^+$ T cell levels before therapy (in $CD4^+$ T cells/ml) ("CD4Base"). (C) Plot of linear regression analysis of the change in viral load ("vlchange") as a function of weekly dose of hCG in IU ("HCGIU"). For all three panels, data points for patients on hCG therapy as well as non-protease and protease inhibitors are represented by open triangles, those on hCG therapy and non-protease inhibitors by open diamonds, and those on hCG alone by solid circles.

Figures 16A and B. (A) Plot of protein concentration (as mAUFS, milli absorbance units, at 280 nm) as a function of the fraction number of the hCG APL™ preparation Superdex 200 fractionation. (B) Plot of protein concentration (as mAUFS, milli absorbance units, at 280 nm) of molecular weight markers of 670 kD, 158 kD, 44 kD, 17 kD and 1.3 kD (as indicated above the plot) as a function of fraction number of a Superdex 200 column run under the same conditions as the fractionation plotted in panel A.

Figures 17A-E. Mass spectrometry profiles of fractions 61, 63, 64, 65, and 67 in panels A-E, respectively.

6. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to proteins (including peptid s) containing a sequence of one or mor portions of β-

hCG (β-hCG peptides) that are effective at inhibiting HIV replication and/or infection *in vitro* or *in vivo*, decreasing viral load, and/or treating or preventing disorders associated with HIV infection. In specific embodiments, the invention provides an isolated protein, the amino acid sequence of which consists of amino acid numbers 41-54, 45-54, 47-53 or 45-57 (SEQ ID NOS:3-6, respectively) of the β-hCG sequence depicted in Figure 8 (a portion of SEQ ID NO:2), particularly to an isolated protein spanning amino acids 45-57 (SEQ ID NO:5) of Figure 8 (a portion of SEQ ID NO:2). The invention also provides isolated proteins comprising or, alternatively, consisting of, the amino acid sequence of two or more portions (preferably non-naturally contiguous portions) of β-hCG, *e.g.*, wherein such portions are linked at the C-termini and N-termini via peptide bond(s). Specifically, the invention provides isolated proteins having an amino acid sequence of β-hCG amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of amino acids β-hCG 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of β-hCG amino acids 110-119 (SEQ ID NO:27); or an isolated protein of β-hCG amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of amino acids β-hCG 108-119 (SEQ ID NO:29) of the β-hCG sequence depicted in Figure 8 (portions of SEQ ID NO:2), *i.e.*, the fused peptides denoted 45-57::109-119, 110-119::45-57, or 47-57::108-119 (SEQ ID NOS:30-32, respectively). The present invention also relates to certain fractions (*i.e.* components of a source of native hCG or native β-hCG isolated away from other components in the source of native hCG or native β-hCG by a separation technique known in the art), particularly fractions from gel filtration sizing chromatography, of a source of native hCG or β-hCG, such as commercial preparations of hCG and human (preferably early, *i.e.* first trimester) pregnancy urine, that have anti-HIV and/or anti-KS activity. Native hCG or native β-hCG refers to naturally occurring hCG or β-hCG, *i.e.* not recombinantly produced.

The present invention further relates to therapeutic methods and compositions for treatment and prevention of disorders associated with HIV infection based on hCG and β-hCG preparations, therapeutically and prophylactically effective fractions of a source of native hCG or native β-hCG, and therapeutically and prophylactically effective β-hCG peptides. The invention provides for treatment of HIV infection by administration of a therapeutic compound of the invention. The therapeutic compounds of the invention include: hCG, β-hCG, therapeutically and prophylactically effective fractions of a source of native hCG or native β-hCG, therapeutically and prophylactically effective β-hCG peptides, related derivatives and analogs of hCG, β-hCG or β-hCG peptides, and nucleic acids encoding β-hCG and β-hCG peptides, and analogs and derivatives thereof. β-hCG peptides which are effective for treatment and prevention of HIV infection can be identified by *in vitro* and *in vivo* assays such as those described in Section 6.3, *infra*.

In a preferred embodiment, a therapeutic composition of the invention comprises a β-hCG peptide, the amino acid sequence of which consists of amino acid numbers 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, or 48-56 (SEQ ID NOS:8-25 or 33-35, respectively) of Figure 8 (a portion of SEQ ID NO:2), particularly a β-hCG peptide which consists of amino acid numbers 41-54, 45-54 or 109-119 (SEQ ID NOS:3, 4, or 7, respectively), most preferably of a β-hCG peptide which consists of amino acid numbers 47-53 (SEQ ID NO:5) or 45-57 (SEQ ID NO:6). In another preferred embodiment, a therapeutic composition of the invention comprises a fusion protein comprising two or more β-hCG sequences (either as non-contiguous or contiguous sequences), *e.g.* having an amino acid sequence of one β-hCG peptide linked via a peptide bond to another β-hCG peptide, in particular a protein, the amino acid sequence of which consists of amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-

terminus of amino acids 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of amino acids 110-119 (SEQ ID NO:27); or an isolated protein of amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 108-119 (SEQ ID NO:29) of the β-hCG sequence depicted in Figure 8 (portions of SEQ ID NO:2), *i.e.*, the peptides denoted 45-57::109-119, 110-119::45-57, or 47-57::108-119 (SEQ ID NOS:30-32, respectively).

In other preferred embodiments, the therapeutic comprises a β-hCG peptide, the amino acid sequence of which consists of circularized (via a disulfide bond between its amino- and carboxy-terminal cysteines) 44-57 (SEQ ID NO:26) with the valine at position 44 substituted with cysteine ((Val44Cys) 45-57 circularized) (depicted in Figure 9B), the circularized (via a disulfide bond between its amino- and carboxy- terminal cysteines) fused peptide of amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus by a peptide bond to the N-terminus of amino acids 45-57 (SEQ ID NO:6), or the peptide 45-57 (SEQ ID NO:6) where the amino acid residues at positions 47 and 51 are substituted by a branch, where the branches are made up of diaminobutyric acid peptide bonded to a proline residue (depicted in Figure 9A). The amino acid sequence of β-hCG is depicted in Figure 8 (SEQ ID NO:2). In yet another embodiment, the therapeutic comprises fractions, preferably fractions of a source of hCG or β-hCG, such as commercial hCG preparations and human early pregnancy urine, of material eluting from a gel filtration column with apparent molecular weights of approximately 40 kD, 15 kD or 2-3 kD as determined based on in which fractions native hCG dimer (77kD) and β-hCG core protein (10 kD) elute.

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections which follow.

6.1. *β-hCG* PEPTIDES AND DERIVATIVES THEREOF

The invention provides isolated proteins (*e.g.*, peptides), the amino acid sequences of which consist of one or more portions of the β-hCG sequence (β-hCG peptides), and derivatives thereof, which are effective for treatment or prevention of HIV infection and resulting disorders. In various specific embodiments, the portions of the β-hCG sequence are at least 3, 5, 10, 20, or 30 amino acids. Effectiveness of the peptides of the invention for treatment or prevention of HIV infection can be determined by any of the methods disclosed in Section 6.3 *infra* or by any method known in the art. In a specific embodiment, the peptides inhibit HIV infection or replication. In a preferred embodiment, the invention relates to proteins, the amino acid sequences of which consist of amino acid numbers 41-54, 45-54, 47-53 and 45-57 (SEQ ID NOS:3-6) of the β-hCG sequence depicted in Figure 8 (a portion of SEQ ID NO:2). In other embodiments, proteins, the amino acid sequences of which consist of amino acid numbers 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, and 48-56 (SEQ ID NOS:7-25 and 33-35, respectively) of Figure 8 (a portion of SEQ ID NO:2) are also provided.

In another embodiment, the invention provides proteins, the amino acid sequences of which consist of two or more at least 5, 7 or 10 amino acid, non-naturally contiguous portions of the β-hCG sequence (Figure 8 (SEQ ID NO:2)) linked by peptide bonds between the N-terminus of one portion and the C-terminus of another portion. Specifically, proteins, the amino acid sequences of which consist of amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of a peptide of amino acids 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of amino acids 110-119 (SEQ ID NO:27); or an isolated protein of amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 108-119 (SEQ ID NO:29) of the β-hCG

sequence depicted in Figure 8 (portions of SEQ ID NO:2), *i.e.*, the fused peptides denoted as 45-57::109-119, 110-119::45-57, or 47-57::108-119 (SEQ ID NOS:30-32, respectively). Derivatives of the foregoing fusion proteins are also provided (*e.g.*, branched, cyclized, N- or C-terminal chemically modified, etc.). In another embodiment, fusion proteins comprising two or more such portions of the β-hCG sequence are provided; such portions may or may not be contiguous to one another (*i.e.*, an intervening sequence may be present). Molecules comprising such portions linked by hydrocarbon linkages are also provided. In another embodiment, the peptides of the invention (i) have an amino acid sequence consisting of no more than 8 peptides of the β-hCG sequence (Figure 8 (SEQ ID NO:2)) and (ii) comprise amino acid numbers 47-53 (SEQ ID NO:5) of β-hCG (Figure 8 (SEQ ID NO:2)).

In another embodiment, the invention provides an isolated protein which protein (a) comprises one or more portions of the amino acid sequence of β-hCG, a peptide consisting of said portion(s) being effective to inhibit HIV infection or replication; and (b) lacks β-hCG amino acids contiguous to said portion(s). In a specific embodiment, the invention provides an isolated protein (a) comprising a β-hCG amino acid sequence consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-56, 47-58, 58-145, 7-40, 46-65 or 48-56 (SEQ ID NOS:3-6, 8-19, 21, 22, 24, or 33-35, respectively) as depicted in Figure 8 (a portion of SEQ ID NO:2); and (b) lacking β-hCG amino acids contiguous to said sequence. Peptides containing the above sequences in which only conservative substitutions have been made are also provided by the present invention, as but one example of peptide derivatives within the scope of the invention. Analogs of the above-mentioned proteins and peptides which have one or more amino acid substitutions forming a branched peptide (*e.g.*, by substitution with an amino acid or amino acid analog having a free amino- or

carboxy-side chain that forms a peptide bond with a sequence of one or more amino acids, including but not limited to prolines) or allowing circularization of the peptide (*e.g.*, by substitution with a cysteine, or insertion of a cysteine at the amino- or carboxy-terminus or internally), to provide a sulfhydryl group for disulfide bond formation, are also provided.

The peptides and derivatives of the invention may also have utility for uses other than treatment or prevention of HIV, for example but not limited to, the uses disclosed in the U.S. Patent Applications filed on even date herewith, entitled "Treatment and Prevention of Cancer by Administration of Derivatives of Human Chorionic Gonadotropin", by Gallo et al., Serial No. _________ (Attorney Docket No. 8769-025); "Methods of Promoting Hematopoiesis using Derivatives of Human Chorionic Gonadotropin", By Gallo et al., Serial No. ___ (Attorney Docket No. 8769-026); "Treatment and Prevention of Wasting Syndrome Based on Administration of Derivatives of Human Chorionic Gonadotropin", Serial No. _________ (Attorney Docket No. 8769-027); and in U.S. Patent Applications entitled "Treatment and Prevention of Cancer by Administration of Derivatives of Human Chorionic Gonadotropin", by Gallo et al., Serial No. 08/709,925, filed September 9, 1996; "Methods of Promoting Hematopoiesis Using Derivatives of Human Chorionic Gonadotropin", by Gallo et al., Serial No. 08/709,924, filed September 9, 1996; and "Treatment and Prevention of Wasting Syndrome Based on Administration of Derivatives of Human Chorionic Gonadotropin" by Gallo et al., Serial No. 08/709,933, filed September 9, 1996, and in the U.S. Patent Applications entitled "Treatment and Prevention of Cancer by Administration of Derivatives of Human Chorionic Gonadotropin", by Gallo et al., Serial No. 08/669,676, filed June 24, 1996; "Methods of Promoting Hematopoiesis Using Derivatives of Human Chorionic Gonadotropin", by Gallo et al., Serial No. 08/669,654, filed June 24, 1996; and

"Treatment and Prevention of Wasting Syndrome Based on Administration of Derivatives of Human Chorionic Gonadotropin" by Gallo et al., Serial No. 08/669,675, filed June 24, 1996, all of which are incorporated herein by reference in their entireties.

In specific embodiments, peptides of less than 50, or less than 25, amino acids are provided.

The invention also relates to derivatives, modifications and analogs of β-hCG peptides. In one embodiment, β-hCG peptide derivatives can be made by altering the β-hCG peptide sequence by substitutions, additions or deletions that provide for therapeutically effective molecules. Thus, the β-hCG peptide derivatives include peptides containing, as a primary amino acid sequence, all or part of the particular β-hCG amino acid sequence including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a peptide which is functionally active. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Conservative substitutions for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Such β-hCG peptide derivatives can be made either by chemical peptide synthesis or by recombinant production from nucleic acid encoding the β-hCG peptide which nucleic acid has been mutated. Any technique for mutagenesis known in the art can be used, including but not limited to, chemical mutagenesis, *in vitro* site-directed

mutagenesis (Hutchinson, C., et al., 1978, *J. Biol. Chem* 253:6551), use of TAB® linkers (Pharmacia), etc.

In addition, β-hCG peptides and analogs and derivatives of β-hCG peptides can be chemically synthesized (*see, e.g.,* Merrifield, 1963, *J. Amer. Chem. Soc.* 85:2149-2156). For example, peptides can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography (*e.g., see* Creighton, 1983, Proteins, Structures and Molecular Principles, W.H. Freeman and Co., N.Y., pp. 50-60). β-hCG peptides can also be synthesized by use of a peptide synthesizer. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; *see* Creighton, 1983, Proteins, Structures and Molecular Principles, W.H. Freeman and Co., N.Y., pp. 34-49). Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the β-hCG peptide. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, α-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, γ-Abu, ε-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, Nα-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

By way of example but not by way of limitation, peptides of the invention can be chemically synthesized and purified as follows: Peptides can be synthesized by employing the N-α-9-fluorenylmethyloxycarbonyl or Fmoc solid phase peptide synthesis chemistry using a Rainin Symphony Multiplex Peptide Synthesizer. The standard cycle used for coupling of an amino acid to the peptid -resin growing chain generally

includes: (1) washing the peptide-resin three times for 30 seconds with N,N-dimethylformamide (DMF); (2) removing the Fmoc protective group on the amino terminus by deprotection with 20% piperdine in DMF by two washes for 15 minutes each, during which process mixing is effected by bubbling nitrogen through the reaction vessel for one second every 10 seconds to prevent peptide-resin settling; (3) washing the peptide-resin three times for 30 seconds with DMF; (4) coupling the amino acid to the peptide resin by addition of equal volumes of a 250 mM solution of the Fmoc derivative of the appropriate amino acid and an activator mix consisting or 400 mM N-methylmorpholine and 250 mM (2-(1H-benzotriazol-1-4))-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) in DMF; (5) allowing the solution to mix for 45 minutes; and (6) washing the peptide-resin three times for 30 seconds of DMF. This cycle can be repeated as necessary with the appropriate amino acids in sequence to produce the desired peptide. Exceptions to this cycle program are amino acid couplings predicted to be difficult by nature of their hydrophobicity or predicted inclusion within a helical formation during synthesis. For these situations, the above cycle can be modified by repeating step 4 a second time immediately upon completion of the first 45 minute coupling step to "double couple" the amino acid of interest. Additionally, in the first coupling step in peptide synthesis, the resin can be allowed to swell for more efficient coupling by increasing the time of mixing in the initial DMF washes to three 15 minute washes rather than three 30 second washes.

After peptide synthesis, the peptide can be cleaved from the resin as follows: (1) washing the peptide-resin three times for 30 seconds with DMF; (2) removing the Fmoc protective group on the amino terminus by washing two times for 15 minutes in 20% piperdine in DMF; (3) washing the peptide-resin three times for 30 seconds with DMF; and (4) mixing a cleavage cocktail consisting of 95% trifluoroacetic acid (TFA), 2.4% water, 2.4% phenol, and 0.2% triisopropysilane with the peptide-resin for two hours, then

filtering the peptide in the cleavage cocktail away from the resin, and precipitating the peptide out of solution by addition of two volumes of ethyl ether.

To isolate the peptide, the ether-peptide solution can be allowed to sit at -20°C for 20 minutes, then centrifuged at 6,000xG for 5 minutes to pellet the peptide, and the peptide can be washed three times with ethyl ether to remove residual cleavage cocktail ingredients. The final peptide product can be purified by reversed phase high pressure liquid chromatography (RP-HPLC) with the primary solvent consisting of 0.1% TFA and the eluting buffer consisting of 80% acetonitrile and 0.1% TFA. The purified peptide can then be lyophilized to a powder.

In a preferred embodiment, the invention provides a peptide with branched amino acids (branched peptide), preferably a branched peptide of amino acids 45-57 (SEQ ID NO:6) with branches occurring at positions 47 and 51, respectively, instead of the Gly and Ala residues normally present. Most preferably, diaminobutyric acid is substituted for the gly and ala residues at positions 47 and 51, respectively, and proline bonded to both diaminobutyric acid residues (45-57 branched) as shown in Figure 9A.

In other specific embodiments, branched versions of the β-hCG peptides listed hereinabove are provided, *e.g.*, by substituting one or more amino acids within the β-hCG sequence with an amino acid or amino acid analog with a free side chain capable of forming a peptide bond with one or more amino acids (and thus capable of forming a "branch").

Branched peptides may be prepared by any method known in the art for covalently linking any naturally occurring or synthetic amino acid to any naturally occurring or synthetic amino acid in a peptide chain which has a side chain group able to react with the amino or carboxyl group on the amino acids so as to become covalently attached to the peptide chain. In particular, amino acids with a free amino side chain group, such as, but not limited to, diaminobutyric acid, lysine, arginine, ornithine, diaminopropionic acid and

citrulline, can be incorporated into a peptide so that an amino acid can form a branch therewith, for example, by forming a peptide bond to the free amino side group, from that residue. Alternatively, amino acids with a free carboxyl side chain group, such as, but not limited to, glutamic acid, aspartic acid and homocitrulline, can be incorporated into the peptide so that an amino acid can form a branch therewith, for example, by forming a peptide bond to the free carboxyl side group, from that residue. The amino acid forming the branch can be linked to a side chain group of an amino acid in the peptide chain by any type of covalent bond, including, but not limited to, peptide bonds, ester bonds and disulfide bonds. In a specific embodiment, amino acids, such as those described above, that are capable of forming a branch point, are substituted for β-hCG residues within a peptide having a β-hCG sequence.

Branched peptides can be prepared by any method known in the art. For example, but not by way of limitation, branched peptides can be prepared as follows: (1) the amino acid to be branched from the main peptide chain can be purchased as an N-α-tert-butyloxycarbonyl (Boc) protected amino acid pentafluorophenyl (Opfp) ester and the residue within the main chain to which this branched amino acid will be attached can be an N-Fmoc-α-γ-diaminobutyric acid; (2) the coupling of the Boc protected amino acid to diaminobutyric acid can be achieved by adding 5 grams of each precursor to a flask containing 150 ml DMF, along with 2.25 ml pyridine and 50 mg dimethylaminopyridine and allowing the solution to mix for 24 hours; (3) the peptide can then be extracted from the 150 ml coupling reaction by mixing the reaction with 400 ml dichlormethane (DCM) and 200 ml 0.12 N HCl in a 1 liter separatory funnel, and allowing the phases to separate, saving the bottom aqueous layer and re-extracting the top layer two more times with 200 ml 0.12 N HCl; (4) the solution containing the peptide can be dehydrated by adding 2-5 grams magn sium sulfate, filtering out the magnesium sulfate, and evaporating the remaining solution to a volume of about 2-5

ml; (5) the dipeptide can then be precipitated by addition of ethyl acetate and then 2 volumes of hexanes and then collected by filtration and washed two times with cold hexanes; and (6) the resulting filtrate can be lyophilized to achieve a light powder form of the desired dipeptide. Branched peptides prepared by this method will have a substitution of diaminobutyric acid at the amino acid position which is branched. Branched peptides containing an amino acid or amino acid analog substitution other than diaminobutyric acid can be prepared analogously to the procedure described above, using the N-F-moc coupled form of the amino acid or amino acid analog.

In a preferred embodiment, the peptide is a cyclic peptide, preferably a cyclic peptide of β-hCG amino acids 44-57 with cysteine substituted for valine at position 44 (SEQ ID NO:26) and circularized via a disulfide bond between the cysteine residues at positions 44 and 57 (C[V44C] 45-57) (Figure 9B), or a cyclic fused peptide of β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus by a peptide bond to the N-terminus of amino acids 45-57 (SEQ ID NO:6) and circularized via a disulfide bond between the cysteine residues at positions 110 and 57. In another preferred embodiment, the peptide is a cyclic branched peptide of β-hCG amino acids 44-57 (SEQ ID NO:26) with cysteine substituted for valine at position 44 and circularized via a disulfide bond between the cysteine residues at positions 44 and 57 and positions 47 and 51 substituted with a diaminobutyric acid residue on which a proline is peptide bonded to its free amino sidechain.

Cyclization can be, for example but not by way of limitation, via a disulfide bond between two cysteine residues or via an amide linkage. For example, but not by way of limitation, disulfide bridge formation can be achieved by (1) dissolving the purified peptide at a concentration of between 0.1.-0.5 mg/ml in 0.01 M ammonium acetate, pH 7.5; (2) adding 0.01 M potassium ferricyanide to the dissolved peptide dropwise until the solution appears pale yellow in

color and allowing this solution to mix for 24 hours; (3) concentrating the cycliz d peptide to 5-10 ml of solution, repurifying the peptide by reverse phase-high pressure liquid chromatography (RP-HPLC) and finally lyophilizing the peptide. In a specific embodiment, in which the peptide does not contain two appropriately situated cysteine residues, cysteine residues can be introduced at the amino-terminus and/or carboxy-terminus and/or internally such that the peptide to be cyclized contains two cysteine residues spaced such that the residues can form a disulfide bridge.

Alternatively, a cyclic peptide can be obtained by generating an amide linkage. An amide linkage can be achieved by, for example, but not limited to, the following procedure: An allyl protected amino acid, such as aspartate, glutamate, asparagine or glutamine, can be incorporated into the peptide as the first amino acid, and then the remaining amino acids coupled on. The allyl protective group can be removed by a two hour mixing of the peptide-resin with a solution of tetrakistriphenylphophine palladium (0) in a solution of chloroform containing 5% acetic acid and 2.5% N-methylmorpholine. The peptide resin can be washed three times with 0.5% N,N-diisopropylethylamine (DIEA) and 0.5% sodium diethyldithiocabamate in DMF. The amino terminal Fmoc group on the peptide chain can be removed by two incubations for 15 minutes each in 20% piperdine in DMF, and washed three times with DMF for 30 seconds each. The activator mix, N-methylmorpholine and HBTU in DMF, can be brought onto the column and allowed to couple the free amino terminal end to the carboxyl group generated by removal of the allyl group to cyclize the peptide. The peptide can cleaved from the resin as described in the general description of chemical peptide synthesis above and the peptide purified by reverse phase-high pressure liquid chromatography (RP-HPLC). In a specific embodiment, in which the peptide to be cyclized does not contain an allyl protected amino acid, an allyl protected amino acid can be introduced into the sequence of the

peptide, at the amino-terminus, carboxy-terminus or internally, such that the peptide can be cyclized.

β-hCG peptides can also b obtained by recombinant expression techniques. (See, *e.g.*, Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, 2d Ed., Cold Spring Harbor, New York, Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K., Vol. I, II). The nucleic acid sequence encoding hCG has been cloned and the sequence determined (Figure 8 (SEQ ID NOS:1 and 2) and Xia, H., 1993, *J. Molecular Endocrinology* June 10; 1993:337-343; Sherman, G.B., 1992, *J. Molecular Endocrinology*, June 6, 1992:951-959; Gieseman, L.K. (ed.), 1991, *Basic and Chemical Endocrinology*, pp. 543-567; Ward et al., 1991, in *Reproduction in Domestic Animals*, 4th ed., P.T. Coppos, ed., pp. 25-80, Academic Press, New York) and can be isolated using well-known techniques in the art, such as screening a library, chemical synthesis, or polymerase chain reaction (PCR).

To recombinantly produce a β-hCG peptide, a nucleic acid sequence encoding β-hCG or a β-hCG peptide is operatively linked to a promoter such that β-hCG or a β-hCG peptide is produced from said sequence. For example, a vector can be introduced into a cell, within which cell the vector or a portion thereof is expressed, producing β-hCG or one or more portions thereof. In a preferred embodiment, the nucleic acid is DNA if the source of RNA polymerase is DNA-directed RNA polymerase, but the nucleic acid may also be RNA if the source of polymerase is RNA-directed RNA polymerase or if reverse transcriptase is present in the cell or provided to produce DNA from the RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in bacterial or mammalian cells. Expression of the sequence encoding β-hCG or the β-hCG peptide can be by any promoter known in the art

to act in bacterial or mammalian cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, *Nature* 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, *Cell* 22:787-797), the HSV-1 (herpes simplex virus-1) thymidine kinase promoter (Wagner et al., 1981, *Proc. Natl. Acad. Sci. USA* 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, *Nature* 296:39-42), etc., as well as the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, *Cell* 38:639-646; Ornitz et al., 1986, *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409; MacDonald, 1987, *Hepatology* 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, *Nature* 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, *Cell* 38:647-658; Adames et al., 1985, *Nature* 318:533-538; Alexander et al., 1987, *Mol. Cell. Biol.* 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, *Cell* 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, *Genes and Devel.* 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, *Mol. Cell. Biol.* 5:1639-1648; Hammer et al., 1987, *Science* 235:53-58), alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, *Genes and Devel.* 1:161-171), beta-globin gene control region which is active in erythroid cells (Mogram et al., 1985, *Nature* 315:338-340; Kollias et al., 1986, *Cell* 46, 89-94), myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, *Cell* 48:703-712), myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, *Nature* 314:283-286), and gonadotropin releasing

hormone gene control region which is active in the hypothalamus (Mason et al., 1986, *Science* 234:1372-1378). The promoter element which is operatively linked to the nucleic acid encoding β-hCG or a β-hCG peptide can also be a bacteriophage promoter with the source of the bacteriophage RNA polymerase expressed from a gene for the RNA polymerase on a separate plasmid, *e.g.*, under the control of an inducible promoter, for example, the nucleic acid encoding β-hCG or β-hCG peptide operatively linked to the T7 RNA polymerase promoter with a separate plasmid encoding the T7 RNA polymerase.

In a less preferred embodiment, peptides can be obtained by proteolysis of hCG or β-hCG followed by purification using standard techniques such as chromatography (*e.g.*, HPLC), electrophoresis, etc.

Also included within the scope of the invention are β-hCG peptide derivatives which are differentially modified during or after synthesis, *e.g.*, by benzylation, glycosylation, acetylation, phosphorylation, amidation, pegylation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. In specific embodiments, the peptides are acetylated at the N-terminus and/or amidated at the C-terminus. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

In another embodiment, the β-hCG or β-hCG peptide derivative is a chimeric, or fusion, protein comprising β-hCG or a functional β-hCG peptide (or two or more portions of β-hCG joined by peptide bond(s)) joined at its amino- or carboxy-terminus via a peptide bond to an amino acid sequence of a different protein. In a specific embodiment, the derivative is a fusion protein comprising the β-hCG sequence, or portions thereof, joined at its amino or carboxy-terminus to an amino acid sequence, or portions thereof, of a chemokine which is therapeutically useful in the treatment of

AIDS, for example, the chemokines MIP-1α, MIP-1β or Rantes (for amino acid sequences of these chemokines see Shall, 1991, *Cytokine* 3:165-183). In one embodiment, such a chimeric protein is produced by recombinant expression of a nucleic acid encoding the protein (comprising a β-hCG-coding sequence joined in-frame to a coding sequence for a different protein). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a chimeric product may be made by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer.

6.2. THERAPEUTIC USES

The invention provides for treatment or prevention of diseases and disorders associated with HIV infection by administration of a therapeutic compound (termed herein "Therapeutic"). Such "Therapeutics" include, but are not limited to: hCG, β-hCG, therapeutically and prophylactically effective fractions of a source of native hCG or native β-hCG, and therapeutically and prophylactically effective β-hCG peptides, *i.e.*, those fractions and peptides which prevent or treat HIV infection (*e.g.*, as demonstrated in *in vitro* and *in vivo* assays described *infra*), and derivatives and analogs thereof, as well as nucleic acids encoding hCG, β-hCG and therapeutically and prophylactically effective β-hCG peptides and derivatives and analogs thereof (*e.g.*, for use in gene therapy). Examples of Therapeutics are those proteins described in Section 6.1 and nucleic acids encoding such proteins and frations of native hCG and native β-hCG described below.

A preferred embodiment of the invention relates to methods of using a Therapeutic for treatment or prevention of HIV infection, preferably HIV-1 infection, in a human subject. In a specific embodiment, the Therapeutic is used for the treatment or prevention of HIV infection in a human

subject that does not suffer from a cancer which secretes hCG or hCG fragments. In another specific embodiment, the Therapeutic is used for the treatment or prevention of HIV infection in a human subject who does not suffer from Kaposi's sarcoma (KS). In the treatment of HIV infection, the Therapeutic of the invention can be used to prevent progression of HIV infection to ARC or to AIDS in a human patient, or to treat a human patient with ARC or AIDS.

In a preferred aspect of the invention, proteins, preferably β-hCG peptides and derivatives are used to treat HIV infection. In particular, proteins, or nucleic acids encoding the proteins, containing an amino acid sequence of one or more portions of β-hCG, preferably containing a sequence from amino acids 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, or 48-56 (SEQ ID NOS:8-25 or 33-35, respectively) of Figure 8 (a portion of SEQ ID NO:2), and preferably containing a sequence from amino acids 41-54, 45-54 or 109-119 (SEQ ID NOS:3, 4 or 7, respectively) of Figure 8 (a portion of SEQ ID NO:2), and most preferably containing a sequence from amino acids 47-53 or 45-57 of Figure 8 (a portion of SEQ ID NO:2), or circular [C44V]45-57 peptide (SEQ ID NO:26), or branched 45-57 (SEQ ID NO:6) peptide, or branched circular [V44C]45-57 peptide are used to treat HIV infection. In another embodiment, a protein is used which contains the amino acid sequence of two or more peptides of different, non-naturally contiguous portions of the β-hCG sequence (Figure 8 (SEQ ID NO:2)) in which the N-terminus of one portion is linked to the C-terminus of another portion by peptide bond(s). In a specific embodiment, a protein is used, the amino acid sequence of which consists of amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of amino acids 110-119 (SEQ ID NO:27); or a protein is used that has an amino acid sequence of amino acids 47-57 (SEQ ID NO:28)

linked at the C-terminus via a peptide bond to the N-terminus of amino acids 108-119 (SEQ ID NO:29) of the β-hCG sequence depicted in Figure 8 (portions of SEQ ID NO:2); *i.e.*, the fused peptides represented as 45-57::109-119, 110-119::45-57, or 47-57::108-119 (SEQ ID NOS:30-32, respectively). In yet another embodiment a protein is used which is a cyclic, fused peptide, particularly a cyclic, fused peptide having a sequence consisting of β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 45-57 (SEQ ID NO:6) and being circularized by a disulfide bond between the terminal cysteines at positions 110 and 57. In another embodiment, a protein is used that (a) comprises a β-hCG amino acid sequence consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-119, 109-145, 7-40, 46-65 or 48-56 (SEQ ID NOS:3-25 or 33-35, respectively) as depicted in Figure 8 (a portion of SEQ ID NO:2) and (b) lacks β-hCG amino acids contiguous to said sequence. In yet another embodiment, a purified derivative of a protein is used to treat or prevent HIV infection, the amino acid sequence of which protein is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 or 48-56 (SEQ ID NOS:3-25 or 33-35, respectively) as depicted in Figure 8 (a portion of SEQ ID NO:2). Other β-hCG peptides, and nucleic acids encoding the peptides, and modifications and derivatives thereof, may have utility in the therapeutic methods of the invention.

In yet another embodiment, a fraction, particularly a size fraction, of a source of native hCG or native β-hCG (*i.e.* naturally occurring, not recombinantly produced, hCG or β-hCG) active in inhibiting HIV infection and replication, particularly a size fraction of approximately 40 kD, 15 kD or 2-3 kD, is used to treat or prevent HIV infection. The

utility of β-hCG peptides and fractions of native hCG and native β-hCG sources may be determined by the *in vitro* and *in vivo* assays described in Section 6.3 *infra* or by any other method known in the art.

Additionally, the present inventors have found that different preparations of native hCG and native β-hCG have variable effects on HIV infection both *in vitro* and *in vivo*. Specifically, the inventors found that among the commercial preparations of (non-recombinant) hCG they investigated, hCG from Fujisawa was the most effective, hCG APL™ (Wyeth-Ayerst) the next most effective, and PREGNYL™ (Organon) the next most effective in inhibiting HIV infection and replication. A highly purified hCG preparation and recombinant β-hCG were found not to be active in inhibiting HIV infection *in vitro*. In fact, the present inventors have shown that specific size fractions of an active hCG preparation (APL™; Wyeth Ayerst) have anti-HIV activity in vitro and anti-KS activity both in vitro and in vivo, as described *infra* in Section 8.

These active fractions were eluted from the gel filtration sizing matrix as or close to (i.e., within 5 fractions (where the fractions are 4 ml fractions using a SUPERDEX™ 200 column which is 26 mm^2 by 60 mm)) the fractions containing or that would contain material that is approximately 40 kD (±8 kD), 15 kD (±3 kD) and 2-3 kD (±2 kD) molecular weight. One skilled in the art would understand that these fractions could be subjected to further size fractionation to further isolate the component of these fractions having the anti-HIV and/or anti-KS activity. Additionally, other methods of fractionation, such as ion-exchange chromatography, affinity chromatography, etc., are well known in the art; those skilled in the art would be able to use any available fractionation techniques to obtain the active fractions from the active hCG preparations and human early (first trimester) pregnancy urine. hCG preparations and fractions of hCG preparations can be screened for efficacy in treating or preventing HIV infection by the

assays described in Sections 6.3, 7 and 8 *infra* or by any method known in the art.

In a specific embodim nt, the invention provides a first composition comprising one or more first components of a second composition comprising native hCG or native β-hCG, said first components being separated from other components of the hCG or β-hCG sample, said first components being active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-hematopoietic activity, and said second composition being active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-hematopoietic activity, and said native hCG or native β-hCG not being purified to homogeneity in said second composition. In particular the invention provides a composition comprising components which have been separated from other components of the native hCG or native β-hCG sample by sizing column chromatography, preferably where the components elute from a gel filtration, preferably a SUPERDEX™ 200, sizing column with an apparent approximate molecular weight of 40 kD, 14 kD or 2-3 kD as determined relative to the elution of a native hCG heterodimer, having a molecular weight of 77 kD, and a β-hCG core protein (β-hCG amino acids 6-40 linked via a disulfide bond to β-hCG amino acids 55-92, as depicted in Figure 8 (SEQ ID NO:2)), having a molecular weight of 10 kD.

In a specific embodiment, the therapeutic method of the invention is carried out as monotherapy, *i.e.*, as the only agent provided for treatment or prevention of HIV. In another embodiment, the Therapeutic is administered in combination with one or more anti-viral compounds, for example, protease inhibitors (*e.g.*, saquinavir) and/or reverse transcriptase inhibitors (*e.g.*, azidothymidine (AZT), lamioridine (3TC), dideoxyinosine (ddI), dideoxycytidine (ddC)). The Therapeutic may also be administered in conjunction with chemotherapy (*e.g.*, treatment with adriamycin, bleomycin, vincristine, vinblastine, doxorubicin and/or Taxol) or other therapies known in the art.

In another embodiment, HIV infection is treat d or prevented by administration of a Th rapeutic of the invention in combination with one or more chemokines. In particular, the Therapeutic is administered with one or more C-C type chemokines, especially one or more from the group RANTES, MIP-1α and MIP-1β.

6.2.1. SOURCES OF hCG AND β-hCG

Native preparations (*i.e.* derived from naturally occurring sources and not recombinantly produced) of hCG and β-hCG can be obtained from a variety of sources. Both hCG and β-hCG are commercially available (*e.g.*, Sigma Chemical Company) and hCG is commercially available in a form suitable for therapeutic use in humans (*e.g.*, from Fujisawa, Wyeth-Ayerst Laboratories (APL™), Organon, Inc. (PREGNYL™) and Serono Laboratories, Inc. (PROFASI™)). hCG is also present at particularly high concentrations in the urine of women in the first trimester of pregnancy ("human early pregnancy urine"). Other sources include, but are not limited to, urine from women in the second and third trimesters of pregnancy, urine from patients with proteinuria, urine from patients having hCG secreting tumors or other cancer patients, and from pituitary glands.

Since the inventors have discovered that different sources of hCG have variable effects on HIV infection and cancer cell growth *in vitro* and *in vivo*, one aspect of the invention relates to assaying preparations of hCG for efficacy in treatment or prevention of HIV infection. The therapeutic effectiveness of hCG preparations and fractions can be tested by the *in vitro* or *in vivo* assays described in Section 6.3 *infra* or by any method known in the art. It is preferable to test the hCG preparation or fraction in an *in vitro* assay, *e.g.*, for HIV replication or transcription from the HIV-1 LTR or *in vivo* in an animal model, such as HIV transgenic mice or SIV infected monkeys, before assaying the preparation in humans.

In a specific embodiment, a preparation comprising hCG is used that contains not only the hCG heterodimer but also peptide fragments thereof, *e.g.*, β chain peptides.

hCG and β-hCG can also be purified, or preferably partially purified, from any source known to contain hCG or β-hCG, *e.g.*, urine from pregnant women, using conventional techniques well-known in the art, such as affinity chromatography. For example, antibodies prepared against hCG or β-hCG can be used to prepare an affinity chromatography column which can be used to purify the proteins by well-known techniques (*see, e.g.*, Hudson & May, 1986, *Practical Immunology*, Blackwell Scientific Publications, Oxford, United Kingdom).

The β-hCG-related proteins are preferably prepared by any chemical or enzymatic synthesis method known in the art, as described *supra* in Section 6.1.

6.2.2. FRACTIONATION OF SOURCES OF hCG

The present inventors have found that the component(s) of a source of hCG having anti-HIV and/or anti-KS activity can be further isolated by fractionation of the source of hCG. The inventors have fractionated the active portions of the commercial hCG preparation APL™ (Wyeth-Ayerst) and human early pregnancy urine as described in Section 8 *infra*. Other sources of hCG include, but are not limited to, urine from women in the second and third trimester of pregnancy, urine from proteinuria patients (both pregnant women with preeclampsia and patients with nephrotic syndromes), urine from patients with hCG secreting tumors, and pituitary glands. However, those skilled in the art will appreciate that any source of hCG or β-hCG having anti-HIV activity and/or anti-KS activity and/or a pro-hematopoietic effect can be fractionated to further isolate the active components. The source of hCG or β-hCG can be fractionated using any technique available in the art for the separation and isolation of molecules, for example but not limited to,

sizing chromatography, ion- xchange chromatography, affinity chromatography, etc.

Briefly, by way of example but not by way of limitation, urine can be prepared for fractionation as follows:

Urine is collected and stored either frozen or refrigerated for not more than two (2) days. Then, sodium azide is then added at a concentration of 1 gram/liter and the sample is stored frozen until sufficient material is collected for the fractionation.

At this point, the urine is thawed over night, the pH adjusted to 7.2 to 7.4 with sodium hydroxide and then centrifuged to remove any precipitate (alternatively, the precipitate can be allowed to sediment, *e.g.*, for 1 hour at room temperature, approximately 75% of the supernatant is decanted, the remainder of the supernatant and the precipitate is centrifuged to pellet the precipitate, and the supernatant decanted and added to the first volume of decanted supernatant). The urine is then filtered through, *e.g.*, a 45 micron filter to remove any remaining particulate matter.

Next, the urine is concentrated using any concentration method available in the art which does not remove higher molecular weight material, *e.g.*, material larger than 3,000 daltons in molecular weight; for example, the material may be concentrated using a Pellicon (Millipore) filtration system with a membrane filter cassette having a molecular weight cut off of 3,000 daltons. Concentration with the Pellicon filtration system using the 3,000 molecular weight membrane filter cut off concentrates 30 liters of urine to 500 ml (i.e., a 60-fold concentration) overnight.

To remove salts and lipids, the concentrate can then be passed over a column containing a large volume of Sephadex G25 resin in 0.05 M ammonium bicarbonate (for example, 250 ml of the concentrate can be passed over a column of approximately 1.7 liters, washing the column with 25% ethanol between runs to remove adsorbed

lipids and glycoprotein). The resulting desalted and delipidated urine concentrate is then lyophilized.

The lyophilized urine material or commercial hCG preparation (or any source of native hCG or β-hCG) is resuspended in either phosphate buffered saline (PBS-- 30 mM sodium phosphate buffer, pH 8.3) or in 0.10 M ammonium bicarbonate at a concentration and in a volume appropriate for the column upon which the sample will be loaded, for example, but not limited to 0.5 grams of protein in 6 ml (i.e., approximately 83 mg/ml). It is within the skill of the skilled artisan to determine the concentration and volume of the sample to be subjected to fractionation.

The sample can then be fractionated by any method known in the art for the separation of proteins. A preferred method is high resolution gel filtration on a Pharmacia pre-packed SUPERDEX™ 200 column (26/60) by HPLC using any available HPLC apparatus, *e.g.*, with a Hewlett Packard 1050 HPLC equipped with a photodiode array detector. The resuspended sample is loaded onto the column in 30 mM phosphate buffer, pH 8.3, and the material can then be eluted from the column with 30 mM sodium phosphate buffer, pH 7.0; 2M NaCl in *e.g.* 4 ml fractions. Fractionation can also be performed using other types of chromatography matrices e.g., heparin, DEAE-cellulose, Sephadex A50, Sephadex G100, phenyl sepharose, or any sizing, ion-exchange, affinity chromatography or any other chromatography matrix available in the art. The column chromatography can also be run using any method available in the art, e.g., standard gravity flow or low pressure chromatography, FPLC, or reverse phase HPLC.

Many separation techniques are known in the art. Those skilled in the art would understand how to apply these known techniques to the fractionation of hCG preparations.

Once the material has been fractionated, any method known in the art, such as but not limited to, those described in sections 6.3, 7, and 8 *infra*, can be used to determine which fractions have anti-HIV activity and/or anti-KS activity and/or a pro-hematopoietic effect.

When fractionating by size, such as fractionation on the SUPERDEX™ 200 column, the apparent molecular weight of material in the fractions can be determined by the relative elution of those fractions compared with the elution of specific hCG and β-hCG species having a known molecular weight or with the elution of known protein size markers. In general, proteins elute from a sizing column as a function of their molecular weight. The elution of, for example, hCG and the β-hCG core protein can be determined by assaying the column chromatography fractions for the presence of hCG and the β-hCG core protein, or any hCG or β-hCG species, by any immunoassay technique available in the art, such as radioimmunoassays (either liquid or solid phase), enzyme-linked assays or ELISA assays.

Antibodies, either polyclonal or, preferably, monoclonal, can be generated against hCG or the β-hCG core protein by any method known in the art. Preparation of monoclonal antibodies against hCG and β-hCG species have been described in the art, *see, e.g.*, O'Connor et al., 1994, *Endocrine Reviews* 15:650-683; Krichevsky et al, 1991, *Endocrinology* 128:1255-1264; and Krichevsky et al., 1988, *Endorcrinology* 123:584-593. For the production of antibodies, various host animals can be immunized by injection with hCG, the β-hCG core protein or any other species of hCG, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. For preparation of monoclonal antibodies, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally

developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, th human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Monoclonal cells lines can then be screened for binding to the particular hCG or β-hCG species using the purified species in any type of immunoassay available in the art (*see, e.g.*, Erlich et al., 1985, *Am. J. Reprod Immunol. Microbiol.* 8:48).

The fractions can then be assayed for the presence of the hCG or β-hCG species using a monoclonal antibody specific for the hCG or β-hCG species. The assay can be performed by any method known in the art. For example, an immunoradiometric assay (IRMA) can be used (Krichevsky et al., 1988, *Endocrinology* 123:584-593). Briefly, the IRMA assay is performed by adsorbing an antibody against the hCG or β-hCG species onto the surface of wells of a microtiter plate by incubation in a coating buffer (0.2 M sodium bicarbonate, pH 9.5) overnight at 4°C. The residual non-specific binding sites are blocked by the addition of a 1% bovine serum albumin solution (with 0.1% sodium azide) to the wells for 3 hours at room temperature, and the wells of the microtiter plate are then washed with deionized water. An aliquot of the fraction in assay buffer (0.01 M sodium phosphate, 0.15 M NaCl, 0.01 M EDTA, 0.1% sodium azide, 0.1% bovine γ-globulin, pH 7.4) is incubated in the wells for 24 hours at room temperature. The sample is then removed and the wells washed with deionized water. A solution of a second antibody specific for the hCG or β-hCG species, which antibody has been iodinated with I^{125}, (approximately 40,000 cpm/well) is incubated in the wells for 24 hours at room temperature. The iodinated antibody solution is removed and the wells washed five times with deionized water. The level of radioactivity in each well is then determined in a scintillation counter which can measure γ-irradiation.

6.2.3. <u>GENE THERAPY</u>

In a specific embodiment, nucleic acids comprising a sequence encoding β-hCG or a β-hCG peptide or fused β-hCG peptides (*i.e.*, two or more β-hCG peptides linked at the N-termini and C-termini via peptide bond(s)), are administered for treatment or prevention of HIV infection, by way of gene therapy. Gene therapy refers to therapy performed by the administration of a nucleic acid to a subject. In this embodiment of the invention, the nucleic acid produces its encoded protein that mediates a therapeutic effect by preventing or treating HIV infection. For example, any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, *Clinical Pharmacy* <u>12</u>:488-505; Wu and Wu, 1991, *Biotherapy* <u>3</u>:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* <u>32</u>:573-596; Mulligan, 1993, *Science* <u>260</u>:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* <u>62</u>:191-217; May, 1993, *TIBTECH* <u>11</u>(5):155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, Current Protocols in Molecular Biology, John Wiley & Sons, NY; and Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY.

In a preferred aspect, the nucleic acid encoding β-hCG or a β-hCG peptide or fused β-hCG peptides or related fusion protein is part of an expression vector that produces β-hCG protein or a β-hCG peptide or fused β-hCG peptides or related fusion protein in a suitable host. In particular, such a nucleic acid has a promoter operably linked to the nucleic acid sequence coding for β-hCG or a β-hCG peptide or fused β-hCG peptides or related fusion protein, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, a nucleic acid molecule is used in which th β-hCG sequences and any other desired sequences are flanked by regions that promote homologous

recombination at a desired site in the genome, thus providing for intrachromosomal expression of β-hCG (Koller and Smithies, 1989, *Proc. Natl. Acad. Sci. USA* 86:8932-8935; Zijlstra et al., 1989, *Nature* 342:435-438).

Delivery of the nucleic acid into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vector, or indirect, in which case, cells are first transformed with the nucleic acid *in vitro*, then administered to the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

In a specific embodiment, the nucleic acid is directly administered *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, *e.g.*, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by infection using a defective or attenuated retroviral or other viral vector (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering it in linkage to a peptide which is known to enter the cell or nucleus, *e.g.*, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see *e.g.*, Wu and Wu, 1987, *J. Biol. Chem.* 262:4429-4432) (which can be used to target cell types specifically expressing the receptors), etc. In a specific embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, *e.g.*, PCT Publications WO92/06180 dated April 16, 1992 (Wu et al.); WO92/22635 dated December 23, 1992 (Wilson et al.); WO92/20316 dated November 26, 1992 (Findeis et al.); WO93/14188 dated July 22, 1993 (Clarke et al.), WO93/20221 dated October 14, 1993 (Young)). In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand

comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, *Proc. Natl. Acad. Sci. USA* 86:8932-8935; Zijlstra et al., 1989, *Nature* 342:435-438).

In a specific embodiment, a viral vector that contains the nucleic acid sequence encoding β-hCG or a β-hCG peptide or fused β-hCG peptides or related fusion protein is used. For example, a retroviral vector can be used (see Miller et al., 1993, *Meth. Enzymol.* 217:581-599). These retroviral vectors have been modified to delete retroviral sequences that are not necessary for packaging of the viral genome. Retroviral vectors are maintained in infected cells by integration into genomic sites upon cell division. The nucleic acid to be used in gene therapy is cloned into the vector, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., 1994, *Biotherapy* 6:291-302, which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., 1994, *J. Clin. Invest.* 93:644-651; Kiem et al., 1994, *Blood* 83:1467-1473; Salmons and Gunzberg, 1993, *Human Gene Therapy* 4:129-141; and Grossman and Wilson, 1993, *Curr. Opin. in Genetics and Devel.* 3:110-114.

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, 1993, *Current Opinion in Genetics and*

Development 3:499-503 present a review of adenovirus-based gene therapy. Bout et al., 1994, *Human Gene Therapy* 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., 1991, *Science* 252:431-434; Rosenfeld et al., 1992, *Cell* 68:143-155; and Mastrangeli et al., 1993, *J. Clin. Invest.* 91:225-234.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., 1993, *Proc. Soc. Exp. Biol. Med.* 204:289-300.) Herpes viruses are other viruses that can also be used.

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including, but not limited to, transfection, electroporation, microinjection, infection with a viral vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see *e.g.*, Loeffler and Behr, 1993, *Meth. Enzymol.* 217:599-618; Cohen et al., 1993, *Meth. Enzymol.* 217:618-644; Cline, 1985, *Pharmac. Ther.* 29:69-92) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the

cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. In a preferred embodiment, recombinant blood cells (*e.g.*, hematopoietic stem or progenitor cells) are administered intravenously. Additionally, epithelial cells can be injected, *e.g.*, subcutaneously, or recombinant skin cells (*e.g.*, keratinocytes) may be applied as a skin graft onto the patient. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

In an embodiment in which recombinant cells are used in gene therapy, a nucleic acid sequence coding for β-hCG or a β-hCG peptide or fused β-hCG peptides or related fusion protein is introduced into the cells such that it is expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells, preferably hematopoietic stem or progenitor cells, are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention.

6.3. DEMONSTRATION OF THERAPEUTIC UTILITY

The Therapeutics of the invention are preferably tested *in vitro*, and then *in vivo* for the desired therapeutic or prophylactic activity, prior to use in humans. Any *in vitro* or *in vivo* assay known in the art to measure HIV infection or production can be used to test the efficacy of a Therapeutic of the invention. By way of example, and not by way of limitation, one could use any of the *in vitro* or *in vivo* assays described in Sections 7 and 8 *infra*.

In an embodiment of the invention, a method of screening a preparation comprising hCG, an hCG alpha or hCG beta chain, fragment or derivative of hCG or said alpha or beta chain, or a fraction of a source of hCG or β-hCG for anti-HIV activity

is provided, which assay comprises assaying said preparation or fraction for the ability to inhibit HIV replication or expression of HIV RNA or protein. In one specific embodiment, the hCG preparation or fraction is assayed by a method comprising measuring HIV-1 p24 antigen levels in cultured hematopoietic cells acutely infected with HIV-1, which cells have been contacted with the preparation or fraction; and comparing the measured HIV-1 p24 antigen levels in the cells which have been contacted with the hCG preparation or fraction with said levels in cells not so contacted with the preparation or fraction, wherein a lower level in said contacted cells indicates that the preparation or fraction has anti-HIV activity. In another specific embodiment, the hCG preparation or fraction is assayed by a method comprising measuring the activity of a reporter gene product expressed from a construct in which the HIV-1 LTR is operably linked to said reporter gene, wherein said construct is present in cells which have been contacted with the preparation or fraction; and comparing the measured expression of said reporter gene in the cells which have been contacted with the preparation or fraction with said levels in such cells not so contacted, wherein a lower level in said contacted cells indicates that the preparation or fraction has anti-HIV activity. In another specific embodiment, the hCG preparation or fraction is assayed by a method comprising measuring HIV-1 derived RNA transcripts or HIV-1 antigen levels in HIV-1 transgenic mice administered the preparation or fraction; and comparing the measured transcript or antigen levels in the mice which have been administered the preparation or fraction with said levels in mice not so administered, wherein a lower level in said administered mice indicates that the preparation or fraction has anti-HIV activity. In yet another specific embodiment, the hCG preparation or fraction is assayed by a method comprising measuring SIV p27 antigen levels in the peripheral blood mononuclear cells of SIV infected monk ys administered the preparation or fraction; and comparing the measured antigen

levels in the monkeys which have been exposed to the preparation or fraction with said 1 vels in monkeys not so administered, wherein a lower level in said administered monkeys indicates that the preparation or fraction has anti-HIV activity.

By way of example, to assay a Therapeutic *in vitro*, one can examine the effect of the Therapeutic on HIV replication in cultured cells. Briefly, cultured hematopoietic cells (*e.g.*, primary PBMCs, isolated macrophages, isolated $CD4^+$ T cells or cultured H9 human T cells) are acutely infected with HIV-1 using titers known in the art to acutely infect cells *in vitro*, such as 10^5 $TCID_{50}$/ml. Then, appropriate amounts of the Therapeutic are added to the cell culture media. Cultures are assayed 3 and 10 days after infection for HIV-1 production by measuring levels of p24 antigen using a commercially available ELISA assay. Reduction in p24 antigen levels over levels observed in untreated controls indicates the Therapeutic is effective for treatment of HIV infection.

Additionally, assays for HIV-1 LTR driven transcription are useful for testing the efficacy of Therapeutics of the invention. Specifically, a reporter gene, *i.e.*, a gene the protein or RNA product of which is readily detected, such as, but not limited to, the gene for chloramphenicol acetyltransferase (CAT), is cloned into a DNA plasmid construct such that the transcription of the reporter gene is driven by the HIV-1 LTR promoter. The resulting construct is then introduced by transfection, or any other method known in the art, into a cultured cell line, such as, but not limited to, the human $CD4^+$ T cell line HUT 78. After exposure of the transformed cells to the Therapeutic, transcription from the HIV-1 LTR is determined by measurement of CAT activity using techniques which are routine in the art. Reduction in HIV-1 LTR driven transcription demonstrates utility of the Therapeutic for treatment and/or prevention of HIV infection.

Exemplary tests in animal models are described briefly as follows: First, a Therapeutic of the invention is administered to mice transgenic for HIV-1, *e.g.*, mice which

have integrated molecular clone pNL4-3 containing 7.4 kb of the HIV-1 proviral genome deleted in the gag and pol genes (Dickie, P., et al., 1991, *Virology* 185:109-119). Skin biopsies taken from the mice are tested for HIV-1 gene expression by RT-PCR (reverse transcription-polymerase chain reaction) or for HIV-1 antigen expression, such as expression of gp120 or NEF, by immunostaining. Additionally, the mice are examined for reduction in the cachexia and growth retardation usually observed in HIV-1 transgenic mice (Franks, R.R., et al., 1995, *Pediatric Res.* 37:56-63).

The efficacy of Therapeutics of the invention can also be determined in SIV infected rhesus monkeys (see Letrin, N.L., and King, N.W., 1990, *J. AIDS* 3:1023-1040), particularly rhesus monkeys infected with SIV_{mac251}, which SIV strain induces a syndrome in experimentally infected monkeys which is very similar to human AIDS (Kestler, H., et al., 1990, *Science* 248:1109-1112). Specifically, monkeys can be infected with cell free SIV_{mac251}, for example, with virus at a titer of $10^{4.5}$ $TCID_{50}$/ml. Infection is monitored by the appearance of SIV p27 antigen in PBMCs. Utility of the Therapeutic is characterized by normal weight gain, decrease in SIV titer in PBMCs and an increase in $CD4^+$ T cells.

Once the Therapeutic has been tested *in vitro*, and also preferably in a non-human animal model, the utility of the Therapeutic can be determined in human subjects. The efficacy of treatment with a Therapeutic can be assessed by measurement of various parameters of HIV infection and HIV associated disease. Specifically, the change in viral load can be determined by quantitative assays for plasma HIV-1 RNA using quantitative RT-PCR (Van Gemen, B., et al., 1994, *J. Virol. Methods* 49:157-168; Chen, Y.H., et al., 1992, *AIDS* 6:533-539) or by assays for viral production from isolated PBMCs. Viral production from PBMCs is determined by co-culturing PBMCs from the subject with H9 cells and subsequent measurement of HIV-1 titers using an ELISA assay for p24 antigen levels (Popovic, M., et al., 1984, *Science* 204:497-500). Another indicator of plasma HIV levels and AIDS

progression is the production of inflammatory cytokines such as IL-6, IL-8 and TNF-α; thus, efficacy of the Therapeutic can be assessed by ELISA tests for reduction of serum levels of any or all of these cytokines. Administration of the Therapeutic can also be evaluated by assessing changes in $CD4^+$ T cell levels, body weight, or any other physical condition associated with HIV infection or AIDS or AIDS Related Complex (ARC). Reduction in HIV viral load or production, increase in $CD4^+$ T cell or amelioration of HIV-associated symptoms demonstrates utility of a Therapeutic for administration in treatment/prevention of HIV infection.

6.4. THERAPEUTIC COMPOSITIONS AND METHODS OF ADMINISTRATION

The invention provides methods of treatment and prevention by administration to a subject in need of such treatment of a therapeutically or prophylactically effective amount of a Therapeutic of the invention. The subject is preferably an animal, including, but not limited to, animals such as monkeys, cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human. In a specific embodiment, the subject is a human not afflicted with a cancer which secretes hCG or hCG fragments and, more particularly, not afflicted with KS.

Various delivery systems are known and can be used to administer a Therapeutic of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the Therapeutic, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a Therapeutic nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered

together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved, for example and not by way of limitation, by topical application, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers.

In another embodiment, the Therapeutic can be delivered in a vesicle, in particular a liposome (*see* Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; *see* generally *ibid.*)

In yet another embodiment, the Therapeutic can be delivered in a controlled release system. In one embodiment, a pump may be used (*see* Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (*see* Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, *J. Macromol. Sci. R v. Macromol. Chem.* 23:61 (1983);

see also Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, thus requiring only a fraction of the systemic dose (*see, e.g.*, Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

In a specific embodiment where the Therapeutic is a nucleic acid encoding a protein Therapeutic, the nucleic acid can be administered by gene therapy methods as described *supra* in Section 6.2.3.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a Therapeutic, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the Therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH

buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tabl ts, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the Therapeutic, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The Therapeutics of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those

derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the Therapeutic of the invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vivo* and/or *in vitro* assays may optionally be employed to help predict optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Doses of, for example but not limited to, at least 15,000 I.U. and up to 45,000 I.U. hCG weekly was effective and well tolerated in humans. Weekly doses of 6,000 I.U. in monkeys and 300-500 I.U. in mice were also effective. Predicted suitable doses of a β-hCG peptide for treatment or prevention of HIV infection include, but are not limited to, 1 to 1000 micrograms per week. Routes of administration of a Therapeutic include, but are not limited to, intramuscularly, subcutaneously or intravenously. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or

biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

7. EXAMPLE: EFFECTS OF hCG, β-hCG AND β-hCG PEPTIDE PREPARATIONS ON HIV-1 INFECTION AND DISEASE

As described herein, we have observed beneficial effects of some preparations of human Chorionic Gonadotropin (hCG) and β-hCG against HIV disease including anti-tumor (Kaposi sarcoma, KS), anti-viral, increase in weight and pro-hematopoiesis effects. Our studies document that the same preparations inhibit KS cell growth *in vitro* and induce apoptosis in a mouse model, inhibit HIV acute infection *in vitro*, down regulate HIV gene expression in 30 of 30 HIV-1 transgenic mice, inhibit SIV replication in 3 of 3 SIV acutely infected macaque monkeys with no evidence of viral resistance, promote normal hematopoiesis (including $CD4^+$ T cell rise), and reverse the wasting seen in these animals. Examples of these effects were also noted in some HIV-positive patients treated with some hCG preparations. The strength of these effects varied among crude hCG preparations, and highly purified hCG did not retain these activities. However, anti-KS, anti-viral, and pro-hematopoietic effects were mimicked by native β-hCG and synthetic peptides of the beta subunit of hCG having amino acid sequences of amino acid numbers 45-57 (SEQ ID NO:6), 109-119 (SEQ ID NO:7), circularized 44-57, where cysteine is substituted for the amino acid at position 44 (SEQ ID NO:26), and peptides of amino acid numbers 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of amino acids 110-119 (SEQ ID NO:27); or a peptide of amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 108-119 (SEQ ID NO:29) of the β-hCG sequence depicted in Figure 8 (portions of SEQ ID NO:2). The peptides having an amino acid sequence of amino acid numbers

7-45, 47-55, 46-65, and 48-56 (SEQ ID NOS:21 and 33-35, respectively) of β-hCG (Figure 8 (SEQ ID NO:2) also exhibit activity in *in vitro* assays.

7.1. EFFECTS OF hCG PREPARATIONS ON HIV-1 TRANSGENIC MICE

The HIV-1 transgenic mice used for this study contain 7.4 kb of foreign DNA, including 5.1 kb of the HIV-1 proviral genome deleted in the gag and pol genes and 2.3 kb of vector (Dickie et al., 1991, *Virology* 185:109-119). The birth weights of mice homozygous for the HIV-1 transgene are normal, but soon the mice uniformly display severe growth retardation (Figure 1A), cachexia, and early mortality from expression of HIV-1 genes (env and regulatory genes) which are highly expressed shortly after birth in homozygotes (Franks et al., 1995, *Pediatric Res.* 37:56-63) (Figure 1B). In addition, these mice develop severe hyperkeratotic skin lesions with marked expression of gp120 and nef genes (Kopp et al., 1993, *AIDS Res. Hum. Retroviruses* 9:267-275; Vasli et al., 1994, *AIDS Res. Hum. Retroviruses* 10:1241-1250).

To examine the effects of hCG preparations on HIV transgenic Tg26 mice, the mothers of 30 neonatal mice were administered a commercial preparation of native hCG (APL™, Wyeth Ayerst) (300-500 I.U.), and the mothers of other HIV-1 transgenic mice received other commercial native hCG preparations (PREGNYL™ and Sigma (G10), α-chain preparations and partially purified native β-hCG and recombinant β-hCG preparations (all Sigma) (50-100 μg). For studies involving synthetic peptides, heterozygous transgenic mothers of 6 homozygous transgenic mice were given 10 μg of β-hCG peptide 45-57 (SEQ ID NO:6) where the amino acid residues at positions 47 and 51 are substituted by a branch, where the branches are made up of diaminobutyric acid and proline (branched β-hCG 45-57) (prepared by Dr. N. Ambulos, UMAB) subcutaneously, daily for 10 days. Other studies were carried out with other β-hCG peptides (see Table 1).

Heterozygous transgenic m thers were treated with the hCG preparation subcutaneously twice weekly. Pups received

hCG via the mother's milk. Blood levels of hCG in the mother and pups were monitored and ranged from 5 IU/ml to over 150 IU/ml over a 96 hour time period (data not shown).

Gene expression was assayed in total RNA extracted from the skin of Tg26 mice with RNAzol. One microgram of RNA was reverse transcribed into cDNA using random hexamer primers and MMTV reverse transcriptase (Life Technologies, MD) in a final volume of 30 μl. One tenth of the cDNA reactions were used for PCR amplification of various HIV gene sequences (<u>env</u>, <u>tat</u>, <u>rev</u>, <u>nef</u> and <u>vif</u>). In addition, glyceraldehyde 3-phosphate dehydrogenase (GAPHD) mRNA was amplified for each sample for normalization. Following 25 cycles of amplification, 10% of the PCR product was resolved by electrophoresis through 2% agarose gels and processed for Southern hybridization using FITC-labeled oligonucleotide probes complementary to internal sequences of the amplicons. Detection was performed by chemiluminescence (Amersham) and relative mRNA levels determined by densitometry after normalization with GAPDH mRNA levels.

The hCG (APL™) treatments resulted in marked down regulation of HIV-1 gene expression in skin biopsies as determined by the RT-PCR technique (Figure 1D). The 25 cycles of amplification employed in these experiments readily detected abundantly expressed genes (*e.g.*, <u>env</u> and <u>rev</u>) while the <u>tat</u> gene in treated animals was at low levels, and more readily detected with more cycles of amplification (not shown). Other skin biopsies were examined for HIV viral proteins using mouse monoclonal antibodies against gp120 and Nef by an immunostaining technique. A marked decrease in viral proteins occurred after 2 weeks treatment and no detectable HIV proteins were found after 30 days of hCG treatment (not shown) and the hyperkeratosis of the skin regressed. When the treatment was halted, reappearance of viral protein expression occurred after 2 weeks (not shown).

Associated with the decrease in HIV-1 gene expression (Figure 1D) was a reversal of the growth retardation and cachexia (Figure 1C). As described immediately below, native

β-hCG and some synthetic peptides of the β-subunit also revers d the adverse effects of the viral genes in these transgenic animals (Table 1). In contrast, native α-hCG had no effect on HIV gene expression or the retarded postnatal growth and cachexia (Table 1).

Table 1 and Figures 1A and B also provides results from the administration of various β-hCG peptides and β-hCG peptide derivatives, *i.e.* the peptides of amino acids 45-57 (SEQ ID NO:6) ("Satellin A1"), circularized 44-57 with cysteine substituted at position 44 (SEQ ID NO:26) ("Satellin A2"), 47-57 linked at the C-terminus via a peptide bond to the N-terminus of 108-119 (SEQ ID NO:32) ("Satellin A1/B^{G}"), 45-57 linked at the C-terminus via a peptide bond to the N-terminus of 109-119 (SEQ ID NO:30) ("Satellin A1/B"), 41-54, 6-16, 47-55 (SEQ ID NO:20), and 48-56 (SEQ ID NO:35). All animals born to HIV-1 transgenic mothers which did not receive preparations containing β-hCG peptide or derivative thereof died within 10 days, showed high level of gp120 and nef protein as measured by antibody staining, and exhibited characteristic hyperkeratosis. The pups receiving the β-hCG peptides 45-57, circularized 44-57 with cysteine substituted at position 44, 47-57 linked by peptide bond to 108-119, 45-57 linked by peptide bond to 109-119 (SEQ ID NOS:6, 26, 32 and 30, respectively) all exhibited considerable inhibition of HIV-1 transcription and protein expression and higher weight gain than untreated mice. The peptides 47-55 and 48-56 (SEQ ID NOS:20 and 32, respectively) also elicited inhibition. The scrambled peptide 45-57::109-119 and the scrambled circularized 44-57, administered as controls, showed no inhibitory effect while, surprisingly, the scrambled 45-57 peptide did exhibit some inhibition (Table 1).

Table 1

ACTIVITIES OF hCG AND hCG SUBUNIT PREPARATIONS AND hCG PEPTIDES.

Sources	HIV in vitro	HIV transgenic mice	KS in vitro	KS in vivo	Pro-hematopoiesis in vitro
	Inhibition				Enhancement
hCG preparations					
APL™	+++	+++	+++	+++	+++
PREGNYL™	++	++	++	++	++
ORGANON	-	ND	-	-	ND
PROFASI™	+	ND	+	+	+
GOLDLINE	+	ND	-	-	-
STERIS™	++	ND	++	ND	++
SHEIN	+	ND	-	-	-
SIGMA	+++	+++	+++	+++	+++
SIGMA[2]	-	ND	-	-	-
CR127	-	ND	-	ND	-
CR1XY17V	-	ND	-	ND	-
CR1XY17B	-	ND	-	-	-
rhCG	-	-	-	-	-
hCG subunits					
α Chain					
αhCG	-	-	-	-	-

Sources	HIV in vitro	HIV transgenic mice	KS in vitro	KS in vivo	Pro-hematopoiesis in vitro
	Inhibition				Enhancement
rαhCG	-	-	-	-	-
αfp1769A	-	ND	-	ND	-
β Chain					
rβhCG	-	-	-	-	-
βhCG	++	++	++	++	++
Synthetic peptides β-chain hCG					
1. 109-119	+	ND	+	+	+
2. 109-145	+	ND	+	+	+
3. 45-57	++	++	++	++	++
4. Circ 44-57	+++	+++	+++	+++	+++
5. 47-57::108-119	++	++	++	+++	ND
6. 45-57::109-119	++	++	++	++	++
7. 45-57+109-119	++	ND	++	ND	++
8. 41-54	-	-	-	-	-
9. 38-57	-	ND	-	-	-
10. Scrambled 45-57::109-119	-	-	-	-	-
11. Scrambled 45-57	++	ND	++	ND	ND

Sources	HIV in vitro	HIV transgenic mice	KS in vitro	KS in vivo	Pro-hematopoiesis in vitro
		Inhibition			Enhancement
12. Scrambled circ. 44-57	-	ND	-	ND	-
13. 6-16	-	-	-	-	-
14. 1-20	-	ND	-	ND	ND
15. 20-47	—	ND	—	ND	—
16. 31-50	-	ND	-	ND	-
17. 46-65	+	ND	+	ND	ND
18. 91-112	ND	ND	-	ND	-
19. 93-100	-	ND	-	ND	ND
20. 110-145	ND	ND	-	ND	-
21. 74-95	-	ND	-	ND	-
22. 7-40	+	ND	+	ND	-
23. 57-93	-	ND	-	ND	-
24. 34-39	-	ND	-	ND	ND
25. 123-145	-	ND	-	ND	ND
26. 134-144	-	ND	-	ND	-
27. 100-110	-	ND	-	ND	ND
28. 113-132	ND	ND	-	ND	-
29. 128-145	-	ND	-	ND	-V

Sources	HIV in vitro	HIV transgenic mice	KS in vitro	KS in vivo	Pro-hematopoiesis in vitro
	Inhibition				Enhancement
30. 37-55	+	+	+	+	+
31. 51-59	-	ND	-	ND	-
32. 48-56	+	+	+	+	+
33. Trimers	-	ND	-	ND	-
Synthetic peptides α-chain hCG					
34. 88-92	-	ND	-	ND	ND
35. 1-15	—	ND	-	-	-
36. 16-30	-	ND	-	ND	-
37. 26-45	-	ND	-	ND	-
38. 41-61	-	ND	-	ND	ND
39. 57-76	-	ND	-	ND	ND
40. 72-92	-	ND	-	ND	-
41. 1-95	-	-	-	-	-

In Table 1, "-" represents less than 10% effect; "+" represents greater than 15% effect; "++" represents greater than 40% effect; "+++" represents greater than 70% effect; and "ND" represents no data available. The "HIV: in vitro" column reports results from assays of the inhibition of HIV-1 replication in vitro (HIV-1 strains and HIV-1 primary isolates) as described infra sections 7.4 and 7.6. The "HIV transgenic mice" column reports data from the inhibition of HIV RNA and protein expression in HIV-1 transgenic mice as described in section 7.1. Columns labeled "KS:in vitro" and "KS:in vivo" report on the inhibition of Kaposi's Sarcoma cell growth in vitro in cultured cells and of Kaposi's Sarcoma induced in mice, respectively, as described in section 7.7. Column 5 provides data on the relative increase of hematopoietic colony cell number in vitro clonogenic assays as described in section 7.8. The commercial hCG preparations tested were APL™ (Wyeth Ayerst), PREGNYL™ (Organon), ORGANON (a highly purified preparation obtained from Organon) PROFASI™ (Serono), Goldline, STERIS™, and Shein, and two preparations from Sigma, Sigma[1] (GHO) and Sigma[2] (C1063). The hCG preparations CR127 and CR1XY17V are highly purified hCG preparations and CR1XY17B is a mixture of highly purified α-hCG and β-hCG, all three preparations were obtained from the National Institute of Child Health and Human Development (NICHD) at the National Institute of Health (NIH) and the rhCG is recombinant hCG expressed in a mouse cell line (Sigma). For the hCG subunits "αhCG" and "βhCG" are purified native subunits (Sigma); "rαhCG" and "rβhCG" all the recombinant subunits expressed in mouse cells (Sigma); and αfp1769A is purified, native α subunit (NICHD, NIH). The peptide "scrambled A1" has the sequence Cys-Val-Ala-Gln-Pro-Gly-Pro-Gln-Val-Leu-Leu-Val-Leu-Cys (SEQ ID NO:36) and "Scrambled A2" has the sequence Cys-Val-Ala-Gln-Gly-Val-Leu-Pro-Ala-Leu-Pro-Gln-Val-Val-Cys (SEQ ID NO:37). "Scrambled A1/B" has the sequence of β-hCG amino acids 45-57 (SEQ ID NO:6) and 109-119 (SEQ ID NO:7) which has been scrambled. "Trimers" is a mixture of tripeptides from the β-hCG sequence

of amino acids 45-57: Leu-Gln-Gly, Leu-Gln-Pro, Gln-Gly-Val, Gln-Pro-Val, Gln-Val-Leu, Val-Leu-Pro, Leu-Pro-Ala, Leu-Pro-Pro, Pro-Ala-Leu, Pro-Pro-Leu, Ala-Leu-Pro, Pro-Leu-Pro, Leu-Pro-Gln, Pro-Gln-Val, Gln-Val-Val, and Val-Val-Cys (SEQ ID NOS: 38-53, respectively). Peptides were synthesized by Dr. N. Ambulos (University of Maryland Biomedicine Center), Becham (CA) or Peptide Technologies Corp. (Gaithersburg, MD).

7.2. EFFECTS OF hCG PREPARATIONS IN SIV INFECTED RHESUS MONKEYS

Events early in HIV infection are thought to be critical to subsequent AIDS pathogenesis. Although the early events in HIV infection are difficult to study in humans, they can be readily investigated in the SIV infected rhesus monkey animal model (Letvin et al., 1990, *J. AIDS* 3:1023-1040). SIV and HIV-1 are similar in many of their biological and physical properties including their genomic structure. In addition, SIV_{mac251}, unlike several other SIV isolates, induces a syndrome in experimentally infected rhesus macaques that is similar to human AIDS (Kestler et al., 1990, *Science* 248:1109-1112).

The effect of the same commercially available hCG preparation (APL™, Wyeth Ayerst), which had been prescreened for anti-viral and anti-KS activity, was studied in five adult male rhesus monkeys who were intravenously inoculated with cell free SIV_{mac251} ($10^{4.5}$ $TCID_{50}$/ml). In all animals, SIV p27 was apparent in plasma 14 days after infection, reaching a maximum by about day 20 (not shown). Treatment with systemic injections (3,000 IU, 2 times weekly) of the active commercial preparation of hCG (APL™), was initiated 3 weeks after SIV inoculation. Two months post-inoculation, the characteristic increase of SIV p27 antigen (Figure 2A), reduction of $CD4^+$ T cells (Figure 2B), and weight loss (Figure 2C) occurred in 2 of 2 untreated infected monkeys. In contrast, the 3 infected monkeys treated with this hCG preparation showed weight gain characteristic of uninfected animals of this age (Figure 2C), a marked decrease in SIV p27

(Figure 2A) and an increase in CD4⁺ T cells to normal 1 vels (Figure 2B). These effects were maintained over the 7 months the animals were followed. These results show that this commercially available hCG preparation can control SIV_{mac251} acute infection, increase $CD4^+$ T cells, and promote weight gain in SIV infected rhesus monkeys and that these effects can be maintained. The animals were followed for 7 months, and no evidence of disease or SIV resistance to the hCG preparation developed.

In Figure 2D, results are shown from 4 uninfected controls: 2 received the hCG preparation and 2 received the diluent without the hCG. There is a slight increase in the $CD4^+$ T cells in the treated animals (increasing from 460 mm^3 to 520 mm^3 and from 470 mm^3 to 650 mm^3) (Figure 2D). The 2 treated animals also showed a 1 to 2 kg weight gain (not shown).

7.3. EARLY STUDIES OF SOME hCG PREPARATIONS IN PATIENTS WITH HIV-1 DISEASE

The incidence of KS is greatly increased in HIV-infected persons (Friedman-Kien et al., 1981, *J. Am. Acad. Dermatol.* 5:468-473). Based on experimental studies of the killing effect of some hCG preparations on KS Y-1 cells, clinical trials with some commercially available preparations of hCG given either intralesionally (Hermans et al., 1995, *Cellular and Molecular Biology* 3:357-364; Gill et al., 1996, *New Engl. J. Med.* 335:1261-1269, Harris, P.J., 1995, *The Lancet* 346:118-119) or systemically to KS patients have shown that cutaneous KS lesions were reduced via cell killing by apoptosis following intralesional inoculation (Lunardi-Iskandar et al., 1995, *Nature* 375:64-68; Hermans et al., 1995, *Cellular and Molecular Biology* 3:357-364; Gill et al., 1996, *New Engl. J. Med* 335: 1261-1269) and induced regression of advanced KS disease treated by systemic delivery.

Clinical trials reported herein were undertaken in Belgium and California to evaluate the anti-KS properties of

systemic hCG therapy with or with out concomitant intralesional therapy. Use of anti-viral protease and non-protease inhibitors was not restricted. A total of 47 patients were enrolled under protocols of compassionate use sanctioned by the Institutional Review Boards of the respective centers. 29 patients were treated in Belgium, either on a protocol to investigate intralesional and systemic treatment of cutaneous KS (n=15), or in the pre-clinical phase of that protocol (n=4), or on compassionate use for systemic KS or HIV infection (n=10). The protocol involved intralesional administration of 500 IU hCG (PREGNYL™) to 4 lesions for 2 weeks, followed by subcutaneous administration of 2,500 IU hCG (PREGNYL™) 5 days per week for 4 to 6 weeks. Additional systemic intramuscular or subcutaneous hCG treatment with either PREGNYL™, APL™, or STERIS™ (one patient) was provided as ongoing therapy in some patients or as part of compassionate use protocols.

A total of 18 patients were treated in California with at least 1 month of follow-up as part of an ongoing protocol to evaluate systemic hCG therapy for cutaneous KS. These patients received either 5000 IU of APL™ subcutaneously 7 days per week, 10,000 IU subcutaneously 3 times per week, or 10,000 IU subcutaneously 7 days per week.

Overall 30 patients were on pre-existing, anti-viral therapy (19 on RT inhibitors and 11 on protease inhibitors), 11 were on no anti-virals and 8 were missing information. One patient, PH-RF, was on 3TC therapy before hCG therapy, and despite poor compliance, had an hCG response for visceral KS and viral load, which declined to undetectable on hCG alone.

Thirty-six patients survived the study, 7 (PH-LFA, PH-DD, PH-PJ, PO-BO, PO-RB, PH-JJ, PH-MH) died either from opportunistic infections or multiple organ failure. The vital status of 1 patient is unknown. Two patients, PH-DD and PH-OJ, discontinued hCG treatment because of cholestasis. PH-DD was on concomitant anti-mycobacterial therapy which was felt to be a contributing factor. PH-OJ had preexisting

cholestasis, which was exacerbated by the hCG treatment with a marked increase in alkaline phosphatase and rise in bilirubin which required hospitalization (PH). These values declined by 2-fold following discontinuation of hCG therapy. These cases raise the possibility that liver toxicity may be a rare complication of hCG therapy.

Early clinical experience with relatively low dose intralesional hCG administration for KS indicated partial or complete regression of treated lesions, including 3 of the first 4 patients in the initial pilot study in Belgium (Hermans et al.,1995, *Cellular and Molecular Biology* 3:357-364) as well as a dose dependent effect between 16% (250 IU) and 83% (2,000 IU) in patients reported from California (Gill et al., 1996, *New Engl. J. Med.* 335:1261-1269), and other cases showing striking clearance of visceral (lung and gastrointestinal) KS in very advanced disease following systemic therapy with hCG APL™ or PREGNYL™ within 1 to 3 months of initiating therapy.

Among the 30 cases with cutaneous Kaposi's Sarcoma, 12 were treated with intralesional followed by systemic therapy in Belgium and 18 with systemic therapy only in California. Complete (2/12, Belgium; 2/18, California) and partial (5/12, Belgium; 4/18, California) responses were observed while progressive disease was noted among 2/12 from Belgium and 10/18 in California. The overall response rate for the study (CR + PR) was 43% (13/30). The response rate in the group administered hCG both intralesionally plus systemicly group was 58%, while the response rate was 33% in the group receiving only the systemic treatment. Among 8 patients with both visceral and cutaneous KS treated in Belgium with very advanced pulmonary or gastric lesions, 3 patients experienced complete remissions, 2 patients exhibited tumor stabilization and 3 progressed, in each case after failure of conventional cytotoxic therapy.

AIDS patients treated with hCG therapy were tested for increases in $CD4^{+}$ T cell levels (in numbers of cells per mm^3) and decrease in viral load by one of the following assays for

determining viral load: NASBA (Louache, et al., 1992, *Blood* 180:2991-2999; Geller, et al., 1985, *Archs. Path. Lab. Met.* 109:138-145), which has a lower detection limit of 4,000 copies; Roche Amplicor, with a lower detection limit of 200 copies; RT-PCR, with a lower detection limit of 100 copies; or TCID assay in which the infection of PBMCs in co-culture is determined (Popovic et al., 1984, *Science* 204:497-500). As viral load was assayed retrospectively, the viral load results were not a factor in guiding choice of therapy or changes in therapy. Each patient served as their own control and change in viral load (0.7 log change between baseline and subsequent post hCG viral load, scored as significant) was the endpoint measurement for this analysis. For analysis of the anti-viral effect, in addition to the 10 patients undergoing with synchronous hCG and other anti-viral therapy, 6 patients were excluded because of a lack of base line viral load or insufficient follow up before hCG therapy was stopped or additional anti-viral therapy was started.

Among the 16 cases, 1 (PH-OJ) experienced a fall in viral load of 0.7 log on 2 successive tests at least 1 month apart while on stable anti-viral therapy (see Figures 3A and B), 11 were non responding and 2 (PH-VE and PHGRX) manifested an increase in viral load of at least 0.7 log after hCG therapy on 2 successive tests at least 1 month apart. As illustrated in Figures 3C and D, another patient (PG-1), initially on hCG alone and classified as non responsive by study criteria (2 consecutive values of 0.7 log decrease in viral load over 1 month) on hCG alone, experienced a steady decline in viral load but the second qualifying >0.7 log viral load drop was measured 2 weeks after non protease inhibitor therapy was begun. Because of this short window, it is likely that this second stable viral load point is accounted for by hCG rather than the newly introduced anti-virals. It is noteworthy that $CD4^{+}$ T cell levels were not significantly altered in this case but, the patient's KS progressed, documenting a dissociation of various hCG ffects.

Among the 6 cases being treated with hCG alone (i.e. without other anti-viral therapies) with analyzable data, all were scored as non responsive to the hCG therapy by the scoring criteria although one case (PG-1) noted above (and illustrated in Figures 3C and D) is a probable responder. An additional patient on hCG alone (PG-8; Figures 3E and F) experienced a sustained fall in viral load of 0.5 log over a 2.8 month period of treatment on hCG alone until KS lesions progressed, at which time hCG therapy was discontinued. Thus of the 7 analyzable patients on hCG alone, 4 exhibited a downward trend in viral load, 2 patients showed an increase in viral load, and 1 patient was stable.

To more fully evaluate all data from patients on hCG alone or with stable antiviral therapy, all eligible data points were plotted, as shown in Figure 15A, indicating the coordinates for each data point pre and post therapy, with values on the line representing no change in viral load. Values are distributed more or less equally above and below the line with no obvious trend to suggest a strong anti viral effect. To evaluate a dose response relationship between hCG and viral load, regression analysis for patients on hCG, alone or with stable antiviral therapy is shown in Figure 15C. There was no detectable effect of higher hCG dose on viral load level (r=-.089, p=0.285, N=147 measurements). An analysis by different CD4 strata did not show any significant trends to suggest that level of immunity impacted the hCG effect.

Among the 22 patients with analyzable $CD4^+$ T cell data, 5 demonstrated a pro-$CD4^+$ T cell effect (PH-VE, PH-RF, PG-9, PG-17, and PG-19) characterized by a 50% rise in $CD4^+$ T cell count sustained over at least a one month period, as demonstrated by plotting the data from at least two patients (PH-VE--Figures 3G and H and PG-17--Figures 3I and J). Of these 5 patients, concomitant stable non protease anti-virals were administered to 2 patients, stable protease inhibitors in 2 cases and hCG preparation alone in 1 case. Thus of the 6 cases with valid $CD4^+$ T cell data on hCG preparation alone,

1 manifested a significant respons . No patient experienced an adverse fall in CD4^{+} T cell on hCG preparation therapy, although patient PH-VE experienced an 0.7 log rise in viral load with a sustained 50% fall in CD4^{+} T cell numbers and a partial anti KS response (Figures 3G and H). Similarly, paient PG-17 experienced a significant rise in CD4^{+} T cells and no change in viral load on hCG therapy alone, yet experienced progression of KS after 2.5 months (Figures 3I and J). All CD4^{+} T cell values (except for 2 patients on hCG alone) were at or above baseline, with the most significant rises in those on concomitant stable protease inhibitor or non protease drugs (Figure 15B). There is no correlation between a change in the CD4^{+} T cells count and the dosage of hCG (r=.101, p=.339, N=92) (data not shown).

Among the 26 patients analyzable for weight gain (patients who started hCG preparation therapy coincident with or shortly after starting other anti-viral therapy were excluded), 14 gained weight, 3 experienced weight loss, and 9 remained stable. There was no correlation between weight change and dosage of hCG (data not shown). There was however a pattern observed in some patients where an initial weight gain was followed by a return to baseline levels while others experienced sustained weight gain over several months.

hCG therapy was well tolerated clinically by patients and there was no evidence for an adverse effect of hCG on viral load or CD4^{+} T cell level. In two cases with advanced HIV disease hCG was discontinued because of coincident cholestasis probably due to other medications in one case and opportunistic infections in the other.

7.4. EFFECTS OF hCG PREPARATIONS ON HIV-1 INFECTION *IN VITRO*

To prepare HIV infected cells for the in vitro assay of hCG preparations and β-hCG peptides and derivatives, primary PBMCs, macrophages, and CD4^{+} T cells isolated from peripheral blood and the H9 human T cell line, were acutely or chronically infected with 8 different HIV-1 strains: 4 cell line adapted viruses, namely the macrophage tropic Ba-L

strain (Gartner et al., 1986, *Science* 233:215-219) and the $CD4^+$ T cell tropic MN, RF, and IIIB strains (10^5 $TCID_{50}$/ml) (Popovic et al., 1984, *Science* 204:497-500; Gallo et al., 1984, *Science* 224:500-503); 2 isolates, Ju1083 and G3, from Nigerian AIDS patients passed once in a $CD4^+$ T cell line (Sub-T1); and 2 primary ("clinical") isolates from AIDS patients from Trinidad which were never passed in any cell line and were used at a titer of 7.5 x 10^4 $TCID_{50}$/ml. In all experiments, HIV-1 (10^5 $TCID_{50}$/ml) was added to the cells (10^6 cells/ml) for a 2 hour incubation after which the virus infected cells were washed with 10 ml phosphate buffered saline (PBS) three times to eliminate extracellular virus. The test inhibitor, *i.e.* the hCG or β-hCG peptide preparation was then added and the cells were incubated with the test inhibiter for 9 to 10 days with serial sampling. Cultures were assayed for p24 antigen on days 3 to 10. The inhibition of HIV production by the active preparations was not due to cell toxicity since, at the concentrations used, there was little or no effect on ^{3}HTdR incorporation, or cell viability as assessed by cell counts and 3-[4,5 dimethylthiazol-2-y], 2,5 diphenyltetrazolium, and bromide thiazoylblue (MTT) assays (data not shown).

There were no significant differences in the results with the different strains of HIV-1 (not shown). For illustrative purposes, the data presented are from the assays with the HIV-1 IIIB infected isolated $CD4^+$ T cells (Figure 4A), IIIB infected PBMCs (Figure 4B) and HIV-1 Ba-L infected isolated macrophages (Figures 4C and D). As shown in Figures 4A-D, the inhibitory effects of the peptides or hCG preparations were approximately the same for macrophage tropic (Figures 4C and D) or T cell tropic strains (Figures 4A and B). Infection with primary isolates showed similar inhibition (data not shown). In contrast to the potent inhibition of acute HIV-1 infection *in vitro* by the active hCG preparations or synthetic peptides (see Section 7.6 below), there was slight or moderate inhibition (20-40%) of virus production (HIV-1 IIIB) from chronically infected $CD4^+$ T

cell lines (not shown). All target cells had similar patterns of inhibition with the expected variation in p24 antigen expression (Figures 4A-D).

As already noted, there is significant variation in the activity of various commercial preparations of native hCG to kill KS tumor cells (Lunardi-Iskandar et al., 1995, *Nature* 375:64-68; Hermans et al., 1995, *Cellular and Molecular Biology* 3:357-364; Gill et al., 1996, *New Engl. J. Med* 335:1261-1269). As shown in Figure 4D, there is similar variation in the anti-HIV activities of these preparations. For example, for the native heterodimer preparations, the most active preparation was usually hCG APL™ (Wyeth Ayerst) and Sigma hCG (CG10) followed by hCG PREGNYL™ (Organon) (Figure 4D and Table 1).

Employing APL™ hCG, there is a dose dependent inhibition of HIV-1 replication (Figure 4A). Surprisingly, there was little or no inhibition with the highly purified native hCG heterodimer preparations CRIXY17B (data not shown) and CR127 (Figures 4C and D) (generously supplied by the National Hormone and Pituitary Program and Center for Population Research, NIH) nor with purified recombinant α- or β-chains (Sigma). However, commercially available hCG (APL™, Wyeth Ayerst) and partially purified native β-hCG (NβhCG) (dissociated from hCG, Sigma) was active while native α-hCG was not (Figures 4A-D and Table 1).

7.5. EFFECT OF hCG PREPARATIONS ON HIV-1 TRANSCRIPTION

To further elucidate the anti-HIV effects of crude hCG preparations, we studied the effect of APL™ hCG on a HIV-1 LTR driven expression of a reporter gene, chloramphenicol acetyltransferase (CAT) (Figures 5A-C). The T-lymphocyte cell line HUT 78 was transiently transfected with the HIV-LTR construct 174WTIICAT by electroporation. 1×10^7 cells were resuspended in 0.4 ml RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), and 20 μg of the test plasmid with 2 μg of the Tat expression vector pDEX/Tat were introduced into the cells by a pulse from a Biorad GenePulser II apparatus of

250 V and 950 μF at 4°C. (Plasmids were the generous gift of Dr. Richard Gaynor, University of Texas Southwestern Medical Center.) Cells were then divided into three aliquots and maintained at 37°C, 5% CO_2 for 40 hours in the presence of drug, or an equal volume of diluent. Transiently transfected cells were harvested, lysed and a standard amount (4 μg) of heat-treated extract was incubated in the presence of 0.6 mM acetyl coenzyme A and 0.1 μCi [^{14}C]chloramphenicol in 0.25 mM Tris, pH 7.9 at 37°C for 1 hour. The amount of acetylated [^{14}C] chloramphenicol converted to acetyl [^{14}C] chloramphenicol was determined by thin layer chromatography in chloroform: methanol 95:5 (v/v) to fractionate the reaction mixture. Results were quantified by phosphorimage analysis on a Molecular Dynamics Phosphor Imager 445 SI. For each assay the amount of acetylated chloramphenicol was determined as a fraction of total [^{14}C] in the sample to determine the activity of the CAT enzyme.

The expression of CAT driven by the HIV-1 LTR was inhibited in a dose-dependent manner such that 78% of normal transcription from the LTR was detected in cells treated with 100 IU/ml hCG (APL™) and 36% of normal transcription was detected in cells treated with 500 IU/ml hCG (APL™) (Figure 5A). hCG had no effect on transcription of the SV-40 driven CAT construct (Figure 5B). The hCG preparation also had no inhibitory or cytotoxic effect on these cells even after 40-hour incubation with 500 IU/ml hCG (Figure 5C), as previously shown (Lunardi-Iskandar, Y., et al., 1995, *Nature* 375:64-68). Results from constructs with point mutations in the enhancer (NFkB), SP-1 and TAR regions of the promoter showed essentially the same response to hCG; therefore, none of these important regulatory elements was demonstrated to be necessary for the response to hCG (not shown). These results are in accord with the transgenic mice results and indicate that at least part of the inhibitory effect of these hCG preparations is on transcription of the HIV-1 provirus.

7.6. EFFECTS OF β-hCG PEPTIDES ON HIV-1 INFECTION

Since it is known that hCG has proteolytic products which may co-purify with the heterodimer or its β-chain, a series of α- and β-subunits and various synthetic peptides were compared to the active preparations of the native heterodimer (Figures 4A-D; see also Table 1). Peptides of the α- and β-subunits (0.05 to 50 nmoles/ml), native α chain preparation, and various crude preparations of the native hCG heterodimer (0.01 to 1,000 IU) and the native β-hCG subunit (100 to 1,000 μg) were tested for effects on HIV-1 replication in acutely infected cells by measuring change in viral antigen levels in response to the above noted preparations (Figures 4A-D and Table 1).

The effect of β-hCG synthetic peptides β-hCG 45-57 (SEQ ID NO:6; "satellin A1"), circularized β-hCG 45-57 with cysteine at position 44 (SEQ ID NO:26; "satellin A2"), the peptide β-hCG 109-119 (SEQ ID NO:7; "satellin B"), and the fused peptides of amino acids 47-57 (SEQ ID NO:28) linked by a peptide bond to the N-terminus of 108-119 (SEQ ID NO:29), and 45-57 (SEQ ID No:6) linked at C-terminus by a peptide bond to the N-terminus of 109-119 (SEQ ID NO:7) all inhibit HIV-1 replication in vitro (Table 1). The first three of these peptides were shown to inhibit HIV infection in a dose dependent fashion (Figures 4A, B and C) and had comparable activity in various cell systems (Figures 4A, B and C) and against various viral strains (not shown), including primary field isolates. Additionally, the β-hCG peptides of amino acids 109-145, 46-65, 7-40, 47-55 and 48-56 (SEQ ID NOS:25, 34, 33, 20 and 35, respectively) also inhibited HIV-1 replication (Table 1). Surprisingly, the "Scrambled Satellin A1", with a sequence of Cys-Val-Gln-Pro-Gly-Pro-Gly-Val-Leu-Leu-Val-Leu-Cys (SEQ ID NO: 36) also had an inhibitory effect (Table 1). Native α-hCG, the α-hCG peptides of 88-92 and 1-95, and several other β-hCG peptides had little or no effect (Figures 4A, B and C and Table 1).

7.7. EFFECTS OF β-hCG PEPTIDES ON KAPOSI SARCOMA CELLS

Neoplastic Kaposi's Sarcoma tumor cells with a characteristic chromosomal abnormality have been reported (Delli-Bovi et al., 1986, *Cancer Res.* 46:6333-6338; Siegal, et al., 1990, *Cancer* 65:492-498; Popescu et al., 1995, *JNCI* 88:450-454) and provide a model system for studying the *in vitro* effects of hCG on KS cells. In our prior studies employing immune deficient mice injected with KS tumor cells, some commercial preparations of native hCG killed KS tumor cells *in vivo* apparently by inducing apoptosis and inhibiting angiogenesis. *In vitro* tumor cell colonies were also suppressed in clonogenic assays by the hCG preparations (Lunardi-Iskandar et al., 1995, *Nature* 375:64-68; Nakamura et al., 1988, *Science* 242:426-430; Ensoli et al., 1989, *Science* 243:223-226; Salahuddin et al., 1988, *Science* 242:430-433; Masood, et al., 1984, *AIDS Res. Hum. Retroviruses* 10:969-976). In the current study, experiments were performed to investigate whether the anti-viral effect of the active peptides (Figures 4A-D and Table 1) correlated with the anti-KS effect of native hCG both *in vitro* in clonogenic assays on cultured KS Y-1 cells and *in vivo* in KS tumors induced in nude mice by injection of cultured Kaposi's Sarcoma cells.

Briefly, the KS Y-1 cells were obtained from mononuclear cells isolated from pleural effusion of an AIDS patient with KS in the lungs. After the depletion of T lymphocytes, monocytes/macrophages and fibroblasts using monoclonal antibodies against CD2, CD3, CD4, CD8, CD10 and CD14 membrane antigens and baby rabbit complement, the cells were cultured in the absence of exogenous growth factors to select for transformed cells. Immunological characterization of the KS Y-1 cells showed that CD34, CD31 and endoglin were expressed. Clonogenic assays were performed by seeding the KS Y-1 or KS-SLK cells in methylcellulose (0.8%, v/v), incubating the cells for 10 days in the presence or absence of the hCG, β-hCG or β-hCG peptide preparations and then counting the

number of well-formed colonies of triplicate wells formed after seeding with 5 X 10^4 cells.

As shown in Figure 6A and Table 1, the peptides used in the assay at a concentration of (50 nmoles/ml) with the strongest anti-viral effects (peptides of amino acids 45-57 (SEQ ID NO:6), cyclic 44-57, with cysteine substituted at position 44 (SEQ ID NO:26), 109-119 (SEQ ID NO:7), 109-145 (SEQ ID NO:25), and 47-57 linked at the C-terminus by a peptide bond to the N-terminus of 108-119 (SEQ ID NO:32), and 45-57 linked at the C-terminus by a peptide bond to the N-terminus of 109-119 (SEQ ID NO:30)) also had the strongest anti-tumor effects (i.e., anti-KS effect) on the two KS neoplastic cell lines. It is notable that the highly purified hCG heterodimer (CR127 2 use data concentrations of nmoles/ml) was inactive, as in the *in vitro* HIV assay. There was no anti-KS effect with the highly purified α- and β-chains and the α-hCG peptides, and other β-hCG peptides showed little or no inhibition in clonogenic assays (Table 1). Again, the "Scrambled Satellin A1" peptide (SEQ ID NO:36) exhibited activity while the others scrambled peptides did not.

The effects of the peptides on KS tumor cells were also evaluated *in vivo* in the KS mouse model. To induce KS tumors in the mice, 1 x 10^6/ml KS Y-1 cells in 50 μl PBS or saline were injected subcutaneously into immunodeficient mice (beige-XID-BNX mice). After one week, tumors ranged in size from 2 mm x 3 mm to 3 mm x 5 mm. Methods for detection of apoptosis (from tissue biopsies) were used, as described in Lunardi-Iskandar et al.(1995, *Nature* 375:64-68). Briefly, the samples were stained *in situ* for the presence of cells with DNA fragmentation. Tissue slides from formalin-fixed tumors were treated with terminal deoxynucleotide transferase for extension of DNA ends (hydroxyl 3′) and incorporation of digoxigenin-11-dUTP according to the manufacturer's instructions (Oncor, Gaithersburg, MD). Anti-digoxigenin antibody conjugated with the enzyme peroxidase allowed

detection of apoptotic cells, which stain brown, whereas viable cells stain blue.

Shown in Figures 6B-E are representative examples of the effects of hCG and the β-hCG peptides on KS Y-1 tumors in mice. One week after injection with the tumor cells, the mice were treated with crude hCG (APL™, Wyeth Ayerst) or with β-chain peptides 45-57 (SEQ ID NO:6) and cyclic 44-57[Cys44] (SEQ ID NO:26). Figures 6B-E show hematoxylin and eosin staining of thin tissue sections of KS Y-1 induced tumors. Compared to the frequent mitotic activity in the controls (Figure 6B), there is evidence of extensive cell death in the tumors of the animals treated with the β-hCG peptides which are comparable to the findings in animals treated with active hCG preparations (Figures 6C-E). Table 1 presents data showing that the β-hCG peptides with an amino acid sequence of 47-57 linked at the C-terminus by a peptide bond to the N-terminus of 108-119 (47-57::108-119; SEQ ID NO:32) and 45-57 linked at the C-terminus by a peptide bond to the N-terminus of 109-119 (45-57::109-119; SEQ ID NO:30) also had significant anti-KS activity. Additionally, β-hCG peptides of amino acid numbers 109-119, 109-145, 47-55 and 48-56 (SEQ ID NOS:7, 25, 20 and 35, respectively) exhibited some anti-KS activity. Other α-hCG and β-hCG peptides showed no activity (Table 1).

As noted above, some AIDS-KS patients treated by intralesional or systemic injection of some preparations of hCG experience regression of tumor lesions of the skin as well as visceral KS (Hermans et al., 1995, *Cellular and Molecular Biology* 3:357-364; Gill et al., 1996, *New Engl. J. Med.* 335:1261-1269). Patients receiving these preparations showed macroscopic regression and flattening of KS lesions. In situ immunostaining specific for apoptosis detection in tumor biopsies showed evidence of apoptosis and/or, histologically, complete absence of the KS tumor after 2-3 weeks of hCG therapy as shown in Figures 6F, G and H, similar to that seen in the experimental mouse model with the active β-hCG peptides. In control KS tumors treated with diluent

only or untreated KS tumor tissues (not shown), there was little evidence of cell death (Figure 6F).

7.8. EFFECTS OF hCG AND β-hCG PEPTIDES ON HEMATOPOIESIS

In addition to the typical decline in $CD4^+$ T cells, cytopenias can occur in HIV infected people affecting one or more hematopoietic lineages associated with deficient progenitor cell growth. This deficiency is often made worse by some of the anti-viral therapies currently in use. In contrast, hCG preparations do not inhibit hematopoiesis.

The effect of hCG preparations and peptides was assayed on hematopoietic progenitor cells *in vitro*. Hematopoietic progenitor cells (2×10^5 cell/ml) were isolated from normal bone marrow and cord blood and seeded in methylcellulose. The following hCG preparations and peptides were used in these clonogenic assays were: hCG (APL™); hCG alpha subunit (Sigma); purified hCG heterodimer CR 127; β-hCG peptide 109-119 (SEQ ID NO:7) (Bachem); β-hCG peptide 45-57 (SEQ ID NO:6); β-hCG peptide 45-57 circularized (44-57 with cysteine substituted for the amino acid at position 44, SEQ ID NO:26); mixture of scrambled β-hCG peptides 45-57 and 109-119; and crude preparation of native β-hCG. Also tested were the peptides 45-57 linked at the C-terminus via a peptide bond to the N-terminus of 109-119 (45-57::109-119; SEQ ID NO:30), 47-55 (SEQ ID NO:20) and 48-56 (SEQ ID NO:35) as well as other hCG, α-hCG and β-hCG preparations and α-hCG and β-hCG peptides (Table 1). The hCG preparations were administered at 200 IU/ml and the β- and α- subunits and peptides were administered at 100 μg/ml. The native commercial preparation of hCG (APL™, Wyeth Ayerst) was pre-tested for anti-HIV and anti-KS activities. Aggregates containing more than 50 cells after 10 days of culture were counted as colonies.

As shown in Figures 7A-C and in Table 1, the growth of hematopoietic progenitors (Lunardi-Iskandar et al., 1989, *Leukemia Res.* 13:573-581) is directly promoted by a commercial preparation of partially purified hCG (APL™, Wyeth Ayerst), partially purified native β-chain, and by the

synthetic peptides, β-hCG peptide 45-57 (SEQ ID NO:6), β-hCG peptide 109-119 (SEQ ID NO:7), circularized 44-57 with cysteine substituted for the amino acid at position 44 (SEQ ID NO:26), the peptide 45-57 linked at the C-terminus via a peptide bond to the N-terminus of 109-119 (45-57::109-119; SEQ ID NO:30), and a mixture of the β-hCG peptides 45-57 and 109-119 (SEQ ID NOS:6 and 7, respectively), but not by the highly purified hCG heterodimer (CR127) nor by the recombinant hCG β-chain or the α-chain preparations. The β-hCG peptides 47-55 and 48-56 (SEQ ID NOS:20 and 35, respectively) also exhibited a pro-hematopoietic effect (Table 1). Additionally, scrambled β-hCG peptides 45-57 and 109-119 as well as other β-hCG peptides showed little inhibition (Table 1). Thus, these results recapitulate the anti-KS and anti-HIV effects. Each activity is chiefly effected by the satellin peptides (45-57 and 109-119; SEQ ID NOS:6 and 7, respectively). A series of other peptides of the α- and β-chain had no effect (data not shown).

7.9. DISCUSSION

New treatment regimens for HIV-1 show that a combination of anti-HIV compounds which target reverse transcriptase (RT) such as azidothymidine (AZT), lamivudine (3TC), dideoxyinosine (ddI), dideoxycytodine (ddC) used in combination with an HIV-1 protease inhibitor, have a far greater effect (2 or more logs reduction) on viral load compared to AZT alone (about 1 log reduction) (Perelson et al., 1996, *Science* 15:1582-1586). However, long-term use of combinations of these chemicals may lead to toxicity, especially to the bone marrow and suppression of $CD8^+$ T cells, which may be essential to the control of HIV via killer cell activity (Blazevic et al., 1995, *AIDS Res Hum Retroviruses* 11:1335-1342) and by the release of suppressive factors, notably the C-C chemokines (Cocchi et al., 1995, *Science* 270:1811-1815). Other concerns in long-term chemical anti-retroviral therapy are the possible development of HIV

mutations with partial or complete resistance (Lange, 1995, *AIDS Res Hum Retroviruses* 10:S77-82) and cost.

The discovery of an anti-KS effect of "hCG" was observed *in vivo* in pregnant Bg-nude mice which did not develop KS as did their male litter mates inoculated at the same time with the KS Y-1 tumor cells. This observation led to clinical trials of intralesional therapy for KS which documented responses in 83% of treated lesions in a dose dependent manner (Hermans et al., 1995, *Cellular and Molecular Biology* 3:357-364; Gill et al., 1996, *New Engl. J. Med.* 335:1261-1269).

As shown herein, some patients treated intralesionally with an hCG preparation for KS were noted to have a reduction in viral load, and *in vitro* human cell culture and *in vivo* animal model data show that some preparations of partially purified hCG, partially purified β-hCG and the active β-hCG peptides and fused β-hCG peptides have anti-viral, anti-KS and pro-hematopoietic effects.

We found considerable activity with some preparations of the partially purified native heterodimer and the native partially purified whole β-chain, whereas recombinant β-hCG (purified) and highly purified native hCG heterodimer (CRIXY17B and CR127) were inactive. Based on our results with the β-hCG peptides and fused β-hCG peptides reported here, we suspect that the lower molecular weight species, active portions of them, or possibly larger fragments which include the active amino acid sequences accompany hCG and the β-chain and are not eliminated by some of the purification procedures, thus retaining the anti-viral, anti-KS and pro-hematopoietic effects, but varying among commercial sources. In this respect, it is noteworthy that, although the clinical effects of some preparations of hCG described here were obtained with two different commercial sources (APL™ and PREGNYL™), one was usually more active in laboratory tests (APL™) at lower concentrations than any other preparation. This hCG preparation, however, also varied from lot to lot in the immunodeficient mouse KS system

(data not shown) despite the fact that id ntical amounts (International Units) were used as assessed by the manufacturer's standard bioassays for the conventional use of hCG.

Consequently, we next began studies with a variety of synthetic peptides, and our results show that all the *in vitro* activities of the preparations of native hCG and the *in vivo* mouse data are mimicked strongly by satellin A1 (β-hCG peptide 45-57 (SEQ ID NO:6)), but not other β- or α-peptides or scrambled 45-57 peptide.

The mechanism of the anti-HIV effect of some preparations of native hCG, and native β-hCG, and of the β fragments appear, at least in part, to be direct. This is suggested by: 1) the *in vitro* inhibition of HIV-1 infectivity of CD4^{+} T cells and macrophages; 2) the inhibition of HIV-1 gene transcription in HIV-1 transgenic mice; 3) the rapid clearance of p27 antigen in the acutely SIV infected monkeys treated with hCG; and 4) the decline of plasma virus in some patients treated with some hCG preparations. However, there was a greater inhibition of *in vitro* infection of cells with various strains of HIV-1 compared to inhibition of HIV-1 production from chronically infected cells suggesting that mechanisms, in addition to inhibition of transcription, are also involved. Indirect effects also cannot be excluded for the anti-SIV/HIV effects observed in the monkey experiments and among responding patients. HIV has anti-hematopoiesis effects (Lunardi-Iskandar et al., 1989, *J. Clin. Invest.* 83:610-615; Louache et al., 1992, *Blood* 180:2991-2999; Geller et al., 1985, *Archs. Path. Lab. Met.* 109:138-145). Based on the findings that hCG and the peptides have pro-hematopoietic effects on progenitors cells of the bone marrow, it is possible that enhanced immune function also may have contributed to the *in vivo* results.

Some preparations of hCG have beneficial effects against the range of core problems associated with HIV-1 infection. In laboratory tests, KS cells were killed and regression occurr d of transplanted KS tumors in mice (Lunardi-Iskandar

et al., 1995, *Nature* 375:64-68). A recent clinical study of escalating dose by intralesional injection of hCG (APL™, Wyeth Ayerst) for cutaneous KS skin lesions demonstrated tumor regression in a dose-dependent manner, with 8% responding at the lowest dose (250 IU, 3 times weekly) and 83% at the highest intralesional dose (2000 IU, 3 times weekly) (Gill et al., 1996, *New Engl. J. Med.* 335:1261-1269). Results described here also showed regression of KS lesions in a substantial proportion of cases including cases treated with systemic therapy, and even regression of newly developed lesions while on hCG therapy when higher doses were given. It is also noteworthy that regression of visceral lesions occurred in several KS patients with advanced KS.

The clinical data reviewed herein illustrate many of the beneficial effects observed in the laboratory pre-clinical studies. Since the protocols were not designed to systematically study the various beneficial effects of some preparations of hCG as a treatment for HIV infection and since there is variability in dose and source of product, the inferences to be drawn are illustrative of the potential for some commercial preparations of hCG or related products in HIV and KS treatment. As reported elsewhere (Hermans et al., 1995, *Cellular and Molecular Biology* 3:357-364; Gill et al., 1996, *New Engl. J. Med.* 335:1261-1269), and confirmed herein, some preparations of hCG induced partial or complete regression of KS lesions in patients treated intralesionally (Hermans et al., 1995, *Cellular and Molecular Biology* 3:357-364) and systemically, including advanced visceral disease. In some HIV-1 positive patients at various stages of HIV infection there was a 0.5 to 2 log reduction in plasma viremia level, and in some cases this effect was sustained with no evidence of development of resistance or toxicity and $CD4^+$ T cell levels increased in some as well. Non-fluid weight gain was a very frequent benefit to patients even with far advanced disease. It is noteworthy that patients such as PH-VE have experienced long term ben fits from hCG therapy

without toxicity over more than 80 weeks of therapy documenting the safety of this therapeutic approach.

8. EXAMPLE: FRACTIONATION OF ACTIVE hCG PREPARATIONS AND HUMAN EARLY PREGNANCY URINE

The present inventors have found that certain commercial preparations of hCG, for example, hCG APL™ (Wyeth-Ayerst), had higher anti-HIV, anti-Kaposi's Sarcoma, anti-wasting and pro-hematopoietic activity than other commercial preparations of hCG (see discussion in section 7 *supra*). Further, the inventors have also shown that highly purified preparations of native and recombinant hCG and β-hCG had no activity against HIV infection or replication or against Kaposi's Sarcoma (see results discussion section 7 *supra*). Accordingly, the inventors postulated that there must be an activity in the hCG commercial preparations that is not the hCG dimer or the β-hCG subunit, responsible for the anti-HIV, anti-Kaposi's Sarcoma, anti-wasting and pro-hematopoietic activities. This section presents results of the fractionation of the APL™ hCG commercial preparation and urine from women in the first trimester of pregnancy ("human early pregnancy urine") which also contains hCG. Particular sizing column chromatography fractions were shown to have activity, thus demonstrating that the active components could be fractionated.

8.1. MATERIALS AND METHODS

Both human early pregnancy urine and the APL™ (Wyeth-Ayerst) hCG commercial preparation were subjected to fractionation. For the human early pregnancy urine, 5 liters of urine were collected from women in the first trimester of pregnancy. Twenty-four hour collections were stored frozen or refrigerated for up to 2 days. Upon delivery of the urine to the laboratory, sodium azide was added at 1 g/liter and the urine frozen until five liters had been collected. At this time, all the urine was thawed overnight, and the pH was adjusted to 7.2-7.4 with NaOH, which causes some

precipitation. The precipitate was allowed to sediment for 1 hour at room temperature, most of the supernatant decanted and the remaining supernatant centrifuged to remove any additional precipitate with that supernatant being added to the first supernatant decanted. Next, the urine was concentrated with a Pellicon (Millipore) filtration system using a membrane cassette with a 3,000 MW cut off, which concentrates the urine approximately 60 to 80 fold. Next, the urine was desalted and delipidated by passing 500 ml of the material at a time through a Sephadex G25 column with a volume of 1.7 liters in 0.05 M ammonium bicarbonate (the column was washed between runs with 25% ethanol to remove absorbed lipids and glycoprotein). The material was lyophilized and stored for further fractionation. The urinary material was then reconstituted in 6 ml of 30 mM sodium phosphate buffer, pH 8.3 fractionation.

For the APL™ hCG, the lyophilized hCG preparation from eleven vials (each vial containing 20,000 IU hCG) was resuspended in 6 ml of 30 mM sodium phosphate buffer, pH 8.3 and filtered twice through the 0.45 μm particle filter. For both the lyophilized urine and the hCG APL™, the prepared sample was then loaded onto a pre-packed SUPERDEX™ 200 HiLoad Column (Pharmacia 26 mm^2 x 60 cm) in the 30 mM sodium phosphate buffer, pH 8.3 and then eluted from the column with a solution containing 30 mM sodium phosphate buffer, pH 7.0 and 2 M NaCl. For the first ten minutes, the column flow rate was 1 ml/minute (due to the viscosity of the hCG APL™ material; this flow rate scheme was also used for the urine material); after the first 10 minutes, the flow rate was 2 ml/minute. The column was run on a Hewlett Packard 1050 HPLC equipped with a photodiode array detector. Four ml fractions were collected and frozen until further analysis.

The protein concentration in each fraction was determined by amino acid analysis. A 50 μl aliquot of alternate column fractions was processed for anlysis by hydrolysis in vapors of 6N HCl with 0.1% phenol at 110°C for 24 hours in a Waters Associates Pico-Tag Workstation (Waters,

Milford, MA). An internal standard, norleucine, was added to all fraction samples before hydrolysis to correct for any losses during hydrolysis or liquid transfer. The hydrolyzed samples were then analyzed on a Beckman Instruments 6300 amino acid analyzer and the data was collected on the PE Nelson Data System (Perkin-Elmer) and transformed using PE Nelson Turbochrome software.

The column fractions were monitored with immunoassays to heterodimeric hCG as well as to the hCG beta core fragment (O'Connor et al., 1994, *Endocrin. Rev* 15:650-683; Krichevsky et al., 1994, *Endocrinology* 134:1139-145; Krichevsky et al., 1991, *Endocrinology* 128:1255-1264; O'Connor et al., 1988, *Cancer Res.* 48:1361-1366; Krichevsky, 1988, *Endocrinology* 128:584-593). These two assays permit placement of two internal standard sizes for the gel filtration column: 70,000 kD (hCG) and 10,000 kD (hCG beta core fragment which is amino acids 6-40 of β-hCG linked via a disulfide bond to amino acids 55-92 of β-hCG). External molecular weight standards were also employed to calibrate the column elution positions. In addition, MALDI-TOF mass spectrometry was used to evaluate the ions observed in certain active fractions. Mass spectrometry did indicate that some peptides separated at anomalous positions, showing that they were being carried by other proteins to earlier elution positions in some cases, or interacting with the column matrix and eluting much later than their molecular size would indicate. For example, 3,000 and 6,000 molecular weight materials eluted from the gel filtration column with material of 14,000 molecular weight while 11,000 molecular weight material eluted with material of approximately 1,000-2,000 molecular weight, hCG and hCG-related molecules eluted at their expected positions.

The fractions were then tested for anti-HIV, anti-KS and pro-hematopoietic activities in vitro. To assay for inhibition of HIV-1 replication in vitro, the HIV-1 IIID viral strain was used to infect PBMCs and PM-1 cells (derived from the HUT-78 T-cell lymphoma cell line) at 10^3 $TCID_{50}$/ml as described in detail in section 7.4 *supra*. The infected cells

were incubated for three days in 100 IU/ml of the hCG APL™ or β-hCG C-Sigma preparations; 50-100 μl per ml of the hCG APL™ or early pregnancy urine fractions; 50 μg/ml β-core protein or α-hCG preparation; 200 IU/ml of the highly purified CR127 hCG preparation; or 100 μl/ml of the circularized β-hCG peptide 44-57 (with cysteine substituted at position 44; SEQ ID NO:26).

To assay for activity against Kaposi's sarcoma cell growth in vitro, the clonogenic assay described in section 7.7 *supra* was used with the KS Y-1 and KS-SLK cultured Kaposi's Sarcoma cell lines. The cells were incubated in 200 IU/ml of commercial hCG preparations; 50 μl/ml of certain fractions from the hCG preparation of early pregnancy urine fractionation; or 100 μg/ml β- and α-hCG chains, β-hCG core protein, β-hCG peptides or LH (leuteinizing hormone).

Pro-hematopoietic activity was assayed in in-vitro clonogenic assays as described in Section 7.8 supra. Cells were assayed for colony formation in the presence of 200 IU/ml hCG APL™ or highly purified hCG preparation CR 127; 100 μl/ml of the fractions of the hCG commercial preparation of early pregnancy urine; or 100 μg/ml β-hCG core protein or cyclized β-hCG peptide of amino acids 44-57 (with cysteine substituted at position 44; SEQ ID NO:26).

Certain fractions were tested for activity in reducing Kaposi's Sarcoma lesions in the Kaposi's Sarcoma mouse model as described in section 7.7 *supra*. In this assay, starting one week after injection of the KS Y-1 cells to induce Kaposi's sarcoma formation, the mice were injected subcutaneously with 100 IU hCG APL™; 200 IU highly purified hCG preparation CR127; 100 μg α-hCG, β-hCG, recombinant β-hCG, LH (luteinizing hormone), or β-core protein; 200 μl of fractions of commercial hCG preparation or early pregnancy urine; or 200 μg cyclized β-hCG peptide of amino acids 44-57 (with cysteine substituted at position 44; SEQ ID NO:26) per day for one week. After one week of the week of treatment with the hCG fractions, the KS lesions were examined for cell apoptosis and regression as described section 7.7 *supra*.

Finally, certain fractions were tested for their ability to increase survival, promote weight gain and reduce HIV-1 gene expression in HIV-1 transgenic mice as described in Section 7.1 *supra*. The mothers the mice were administered 300 IU hCG APL™ by osmotic pump or 200 IU hCG APL™ by slow release; 200 μl of certain fractions of hCG commercial preparation or of early pregnancy urine; 200 μg of the cyclized β-hCG peptide of amino acids 44-57 (with cysteine substituted at position 44; SEQ ID NO:26) or the fused peptide of amino acids 45-57::109-119 (SEQ ID NO:30); or 100 μg β-hCG core peptide or the α-hCG sub unit per day, and the pups were dosed through the mother's milk.

The unfractionated APL™ hCG preparation, PREGNYL™ (Organon) hCG preparation, purified β-core and phenol were also tested in certain assays. Phenol, which is an additive in the hCG APL™ preparation, was tested to control for any effect on cell growth or viral inhibition.

8.2. RESULTS

Fractionation of both the APL™ hCG preparation and the human early pregnancy urine resulted in a significant protein peak at approximately 158 kD with diminishing, but still detectable, protein in the rest of the fractions, even those containing small molecular weight material (Figures 10A and D). Fractions containing the hCG dimer (77 kD) and the β-hCG core (10 kD) were identified by immunoprecipitation using antibodies that specifically recognize these particular species, as described in the materials and methods section 8.1. The elution profile of the commercial hCG material was also plotted in comparison to the elution of standard molecular weight markers (Figures 16A and B). Additionally, Fractions 61, 63, 64, 65 and 67 from the fractionation of the commercial hCG material was analyzed by MALDI-TOF mass spectrometry (Figures 17A-E, respectively).

8.2.1. EFFECT OF FRACTIONS OF COMMERCIAL hCG PREPARATIONS AND EARLY PREGNANCY URINE ON HIV-1 REPLICATION IN VITRO

The fractions of both the APL™ hCG preparation and the human early pregnancy urine were assayed for inhibition of HIV-1 IIID replication in PBMCs and PM-1 cells as described in Section 7.4 *supra*. Many of the APL™ hCG preparation fractions exhibited significant inhibition of HIV-1 IIID replication (Figure 10C). In particular, fractions containing material of approximately 70 kD to approximately 2-3 kD exhibited HIV-1 inhibitory activity. The fractions effecting the highest percent inhibition of HIV-1 replication were fractions 62, 63, 65, and 73, with the three main peaks of activity eluting with apparent molecular weights of approximately 40 kD, approximately 15 kD, and approximately 2-3 kD, as determined by comparison with the elution of hCG (77 kD) and β-core protein (10 kD).

The fractions of human early pregnancy urine were also assayed for ability to inhibit HIV-1 IIID replication in the PBMCs and the PM-1 cells. Again, several fractions had at least some HIV-1 replication-inhibitory activity. Fractions 64 and 67 caused more than twice the inhibition of HIV-1 IIID replication than any of the other fractions (Figure 10F). There were approximately two peaks of activity eluting from the gel filtration column with apparent molecular weights of approximately 15 kD and 3 kD, as determined by comparison with the elution of hCG (77 D) and β-core protein (10 kD) identified by immunoassay.

Additionally, phenol had no effect on HIV-1 replication, demonstrating that the anti-HIV activity of the APL™ hCG is not due to the presence of phenol in the APL™ hCG preparation, and purified β-hCG core protein (the peptide of amino acids to 6-40 of β-hCG linked via a disulfide bond to the peptide of amino acids 55-92 of β-hCG as depicted in Figure 8 (SEQ ID NO:2)) was also found not to inhibit HIV-1 replication (data not shown).

8.2.2. EFFECT OF FRACTIONS OF COMMERCIAL hCG AND EARLY PREGNANCY URINE ON KAPOSI'S SARCOMA CELL GROWTH IN VITRO

The fractions of APL™ hCG and human early pregnancy urine were also tested for inhibition of the proliferation of cultured Kaposi's Sarcoma cells. Figure 10B depicts the results of assays of the APL™ hCG fractions for inhibition of KS Y-1 cell growth. There were three major peaks of KS cell growth inhibitory activity which eluted from the gel filtration column with apparent molecular weights of approximately 40 kD, approximately 15 kD, and approximately 2-3 kD, as compared with the elutions of fractions containing hCG dimer (77 kD) and β-core protein (10 kD). A fraction containing material about the same size as the β-hCG core protein exhibited the highest level of inhibition; however, purified β-hCG core was found not to inhibit KS cell growth (data not shown).

Fractions of human early pregnancy urine were also assayed for inhibition of KS Y-1 cell growth. Fractions containing material which eluted from the gel filtration column with apparent molecular weights of approximately 15 kD and approximately 2-3 kD as compared with the elution of fractions containing hCG dimer (77 kD) and the β-hCG core (10 kD) as identified by immunoprecipitation assay were the most effective at inhibiting KS cell growth, with the approximately 15 kD fractions having the highest activity (Figure 10E).

Figure 11 presents additional data on the inhibitory effects of hCG and hCG-related preparations in KS cultured cell clonogenic assays using both the KS Y-1 and KS SLK assays. Fraction 65 (from the peak eluting with an apparent molecular weight of approximately 15 kD) and 76 (from the peak eluting with an apparent molecular weight of approximately 2-3 kD) from the fractionation of both the APL™ hCG preparation (fraction 65 and 76 are represented by bars 12 and 13, respectively,) and the early pregnancy urine (fraction 65 and 76 are represented by bars 10 and 11,

respectively) inhibited growth of both cell lines. The fractions containing material eluting with an apparent molecular weight of approximately 2-3 kD (i.e. fraction 76 of both fractionations) inhibited KS cell growth marginally more effectively than the fractions containing material eluting with an apparent molecular weight of approximately 15 kD (i.e. fraction 65 of both fractionations). Although the active fractions elute close to the fractions containing the β-hCG core protein, purified β-hCG core protein (bar 5) exhibited almost no inhibition of KS cell growth.

The results confirm that the APL™ hCG commercial preparation (bar 1) inhibited KS cell growth better than the other commercial hCG preparations (bars 2-4). Additionally, while native β-hCG (bar 6) inhibited KS cell growth moderately well, α-hCG, the highly purified hCG preparation CR 127 and recombinant hCG (Sigma) (bars 7-9, respectively) inhibit the KS cell growth negligibly. The results also confirm that the cyclized β-hCG peptide of amino acids 44-57 (cysteine substituted at position 44; SEQ ID NO: 26) also inhibited KS cell growth.

8.2.3. EFFECT OF COMMERCIAL hCG AND EARLY PREGNANCY URINE FRACTIONS ON HEMATOPOIESIS IN VIVO

Figure 12 presents data on the effect of hCG and hCG-related preparations on hematopoiesis in in vitro clonogenic assays for numbers of colony forming units of granulocytes, erythrocytes, megakaryocytes and monocytes (CFU-GEMM), burst forming units of erythrocytes (BFU-e) and colony forming units of granulocytes and macrophages (CFU-GM). Figure 12 shows that fraction 65 of both the hCG APL™ and early pregnancy urine fractionation (bars 7 and 8, respectively; fraction 65 contains material with an apparent molecular weight of approximately 15 kD) promoted hematopoiesis in all three assays. Fraction 26 of the early pregnancy urine fractionation (bar 9) did not promote hematopoiesis in any of the assays. The purifi-d β-core protein (bar 10) likewise exhibited no stimulation of hematopoiesis.

These results also confirm that the hCG APL™ preparation, native β-hCG and the cyclized β-hCG peptide of amino acids 44-57 (cysteine substituted at position 44; SEQ ID NO: 26) (bars 3, 5 and 6, respectively) all have pro-hematopoietic activity. The α-subunit of hCG, highly purified hCG preparation CR127 and PBS alone (bars 2, 4 and 1, respectively) did not promote hematopoiesis.

8.2.4. EFFECT OF COMMERCIAL hCG AND EARLY PREGNANCY URINE FRACTIONS ON KAPOSI'S SARCOMA IN VIVO

Certain fractions of the APL™ hCG and early pregnancy urine were assayed for their ability to elicit apoptosis in Kaposi's Sarcoma lesions induced by injection of KS Y-1 cells in mice (n=3 mice for each treatment). The mice were administered 100 μl subcutaneously of the particular fraction each day for one week. Table 2 presents data on the size of the Kaposi's Sarcoma lesions and the percentage of apoptotic cells within the lesion after one week of treatment with fractions 60, 64, 64, 74, 82 and 85 of the APL™ hCG fractions and the unfractionated APL™ hCG preparation. The negative control treated with no hCG or fractionated hCG material exhibited little cell apoptosis or Kaposi's Sarcoma lesion regression (Table 2). Treatment with fractions 82 and 85 (containing material with apparent molecular weights smaller than approximately 2-3 kD) of the APL™ hCG material also elicited almost no Kaposi's Sarcoma lesion regression or apoptosis (Table 2). The unfractionated APL™ hCG, as well as fractions 60 and 74 (fractions within the peaks containing material with apparent molecular weight of approximately 15 kD and 2-3 kD, respectively) of the APL™ hCG fractionated material, caused about 50% apoptosis within the lesion and significant lesion regression (Table 2). Moreover, fractions 64 and 65 (within the peak containing material with apparent molecular weight of approximately 15 kD) of the APL™ hCG showed even higher percentage of apoptosis and more significant lesion regression than the unfractionated APL™ (Table 2).

Additionally, Figure 13 presents results on the effects of certain fractions of the APL™ hCG and the early pregnancy urine on KS tumors induced in mice. Those fractions from the anti-HIV and anti-KS (in vitro) peaks containing material having apparent molecular weight of approximately 15 kD (fraction 65 of the early pregnancy urine ("HAF-UF#") and fractions 62 and 65 of the APL™ hCG preparation ("HAF-CF#")) and of approximately 2-3 kD (fraction 76 of the early pregnancy urine and fractions 74 and 76 of the APL™ hCG preparation) diminished KS tumors in mice as well or better than the unfractionated APL™ hCG ("APL"). However, the fractions tested that were outside these peaks of anti-HIV and anti-KS (in vitro) activities, *i.e.*, fraction 35 of the APL™ hCG (having an apparent molecular weight much larger than the hCG dimer (77 kD)) and fractions 26 and 82 of the early pregnancy urine (having apparent molecular weights much larger than the hCG dimer and smaller than 2-3 kD, respectively) did not cause tumor regression in the mouse model.

Thus, these results correlate with the results from the HIV replication and KS clonogenic assays, that the activity elutes from the gel filtration column in peaks with apparent molecular weights of approximately 15 kD and 2-3 kD (fractions, with an apparent molecular weight of approximately 44 kD were not assayed).

TABLE 2

Fraction	Kaposi's Sarcoma Lesion Size After Treatment (mm x mm)	Percentage Apoptosis Within Lesion
None	14x10,12x17,13x16	3%,2%,5%
APL#60	4x3,3x2,2x2	>50%
APL#64	1x2,1x3,2x3	>60%
APL#65	2x4,2x3,2x1	>60%
APL#74	3x5,2x5,3x4	>50%
APL#82	15x16,13x19,16x14	2%,4%,6%
APL#85	11x24,13x16,10x13	5%,6%,4%
APL prep	2x3,3x3,3x5	>50%

8.2.5. EFFECT OF FRACTIONS OF A COMMERCIAL hCG PREPARATION AND EARLY PREGNANCY URINE ON hCG GENE EXPRESSION IN HIV-I TRANSGENIC MICE

Finally, fractions from the APL™ hCG and early pregnancy urine fractionation were tested for their ability to improve survival and to reduce HIV-I gene expression in HIV-I transgenic mice. Figure 1E presents results of administration of 200 μl of fraction 61 of the APL™ hCG fractionation (bar 4) and fraction 65 of the early pregnancy urine fractionation (bar 5). These two fractions, both of which are within the peak of anti-HIV and anti-KS activity that contains material with an apparent molecular weight of approximately 15 kD, significantly suppressed HIV-I gene expression in the HIV-I transgenic mice (as measured in the skin and the kidney) in comparison to PBS alone (bar 1), β-hCG core peptide (bar 2) and α-hCG subunit (bar 3).

HIV-I transgenic pups administered hCG and hCG-related preparations for the first 5 days after birth through the mothers milk were monitored for survival at 10 days after birth. All the pups receiving the unfractionated APL™ hCG (bar 2), fraction 65 from both the APL™ hCG fractionation and the early pregnancy urine fractionation (bars 4 and 5, respectively), and fraction 76 of the early pregnancy urine fractionation (bar 6) survived (figure 14). These fractions 65 and 76 are within the anti-HIV and anti-KS activity peaks containing material with apparent molecular weights of approximately 15 kD and 2-3 kD, respectively. None of the pups receiving fraction 26 of either the APL™ hCG or the early pregnancy urine (bars labelled as "HAF-CF#26" and "HAF-UF#26", respectively) survived (figure 14). These fractions contain material with an apparent molecular weight larger than the hCG heterodimer (77 kD). Figure 14 also presents data showing that native β-hCG (bar 1) and the β-hCG peptide of amino acids 44-57 (with cysteine substituted at position 44; SEQ ID NO: 26--bar 7) and to a lesser extent LH (bar 3)

promote survival of the HIV-I transgenic pups while the α-hCG subunit, the highly purified hCG preparation CR127, recombinant β-hCG and the β-hCG core protein (bars labelled as "ahCG", "CR127", "rβhCG", and "b-core", respectively) failed to promote survival of the transgenic mouse pups.

8.3. CONCLUSION

The above-described experiments demonstrate that the factor(s) responsible for the anti-HIV and anti-KS activities can be further isolated from the hCG preparations by gel filtration on a SUPERDEX™ 200 gel filtration column. The factor(s) were fractionated from both the commercial APL™ hCG preparation and urine from women in early pregnancy (first trimester). The fractions of highest anti-HIV and anti-KS activity contained material eluting from the gel filtration column with an apparent molecular weights of approximately 40 kD, 15 kD and 2-3 kD. Although certain active fractions contained material of approximately the size of the β-hCG core protein (~10 kD), purified β-hCG core protein was found to have neither anti-HIV nor anti-KS activity. The fractions exhibiting anti-HIV and anti-KS activity in vitro also had pro-hematopoietic activity in vitro, caused regression of KS tumors induced in mice, and promoted survival and reduced HIV-I gene expression in HIV-I transgenic mice. Furthermore, phenol, an additive in the APL™ hCG preparation, had no anti-HIV activity.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various references are cited herein, the disclosures of which are incorporated by reference in their entireties.

SEQUENCE LISTING

(1) GENERAL INFORMATION

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(ii) TITLE OF THE INVENTION: TREATMENT AND PREVENTION OF HIV INFECTION BY ADMINISTRATION OF DERIVATIVES OF HUMAN CHORIONIC GONADOTROPIN

(iii) NUMBER OF SEQUENCES: 37

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(v) COMPUTER READABLE FORM:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 539 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 26..520

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AGACAAGGCA GGGGACGCAC CAAGG ATG GAG ATG TTC CAG GGG CTG CTG CTG       52
                            Met Glu Met Phe Gln Gly Leu Leu Leu
                            -20                 -15

TTG CTG CTG CTG AGC ATG GGC GGG ACA TGG GCA TCC AAG GAG CCG CTT      100
Leu Leu Leu Leu Ser Met Gly Gly Thr Trp Ala Ser Lys Glu Pro Leu
    -10                 -5                  1

CGG CCA CGG TGC CGC CCC ATC AAT GCC ACC CTG GCT GTG GAG AAG GAG      148
Arg Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu Ala Val Glu Lys Glu
                10                  15                  20

GGC TGC CCC GTG TGC ATC ACC GTC AAC ACC ACC ATC TGT GCC GGC TAC      196
Gly Cys Pro Val Cys Ile Thr Val Asn Thr Thr Ile Cys Ala Gly Tyr
            25                  30                  35

TGC CCC ACC ATG ACC CGC GTG CTG CAG GGG GTC CTG CCG GCC CTG CCT      244
Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro
        40                  45                  50

CAG GTG GTG TGC AAC TAC CGC GAT GTG CGC TTC GAG TCC ATC CGG CTC      292
Gln Val Val Cys Asn Tyr Arg Asp Val Arg Phe Glu Ser Ile Arg Leu
    55                  60                  65

CCT GGC TGC CCG CGC GGC CTG AAC CCC GTG GTC TCC TAC GCC GTG GCT      340
Pro Gly Cys Pro Arg Gly Leu Asn Pro Val Val Ser Tyr Ala Val Ala
70                  75                  80                  85

CTC AGC TGT CAA TGT GCA CTC TGC CGC CGC AGC ACC ACT GAC TGC GGG      388
Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg Ser Thr Thr Asp Cys Gly
                90                  95                  100

GGT CCC AAG GAC CAC CCC TTG ACC TGT GAT GAC CCC CGC TTC CAG GAC      436
Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp
            105                 110                 115

TCC TCT TCC TCA AAG GCC CCT CCC CCC AGC CTT CCA AGC CCA TCC CGA      484
Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg
        120                 125                 130

CTC CCG GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA TAAAGGCTTC           530
Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln
    135                 140                 145

TCAATCCGC                                                            539
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 165 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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-20                 -15                 -10                  -5

Gly Thr Trp Ala Ser Lys Glu Pro Leu Arg Pro Arg Cys Arg Pro Ile
                    1                5                   10

Asn Ala Thr Leu Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr
        15                  20                  25

Val Asn Thr Thr Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val
    30                  35                  40

Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg
45                  50                  55                      60

Asp Val Arg Phe Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Leu
                65                  70                  75

Asn Pro Val Val Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu
            80                  85                  90

Cys Arg Arg Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu
        95                  100                 105

Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro
    110                 115                 120

Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr
125                 130                 135                     140

Pro Ile Leu Pro Gln
                145
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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1               5                   10
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Leu Gln Gly Val Leu Pro Ala Leu Pro Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Gly Val Leu Pro Ala Leu Pro
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular, linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro
1               5                   10
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(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro
1               5                   10
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(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro
1               5                   10
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(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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Val Leu Gln Gly Val Leu Pro Ala Leu Pro
1               5                   10
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(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Val Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Leu Gln Gly Val Leu Pro Ala Leu Pro
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gln Gly Val Leu Pro Ala Leu Pro
1 5

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Leu Gln Gly Val Leu Pro Ala Leu Pro Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Gly Val Leu Pro Ala Leu Pro Gln
1               5
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Gly Val Leu Pro Ala Leu Pro Gln Val
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gly Val Leu Pro Ala Leu Pro Gln Val Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg Asp Val Arg

```
1               5                   10                  15
Phe Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Leu Asn Pro Val
            20                  25                  30

Val Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg
        35                  40                  45

Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp
    50                  55                  60

Asp Pro Arg Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser
65                  70                  75                  80

Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu
                85                  90                  95

Pro Gln
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 88 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Asn Tyr Arg Asp Val Arg Phe Glu Ser Ile Arg Leu Pro Gly Cys Pro
1               5                   10                  15

Arg Gly Leu Asn Pro Val Val Ser Tyr Ala Val Ala Leu Ser Cys Gln
            20                  25                  30

Cys Ala Leu Cys Arg Arg Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp
        35                  40                  45

His Pro Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Ser Ser
    50                  55                  60

Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro
65                  70                  75                  80

Ser Asp Thr Pro Ile Leu Pro Gln
                85
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro
1               5                   10                  15
```

Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr
20 25 30

Pro Ile Leu Pro Gln
35

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Cys Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser

```
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Thr Cys Asp
 1               5                  10                  15
Asp Pro Arg Phe Gln Asp Ser Ser
            20
```

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Leu Gln Gly Val Leu Pro
 1               5                  10                  15
Ala Leu Pro Gln Val Val Cys
            20
```

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Leu Thr Cys Asp Asp
 1               5                  10                  15
Pro Arg Phe Gln Asp Ser Ser
            20
```

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu Ala Val Glu Lys Glu Gly
```

```
 1                    5                        10                      15
Cys Pro Val Cys Ile Thr Val Asn Thr Thr Ile Cys Ala Gly Tyr Cys
            20                       25                       30
Pro Thr
```

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg Asp
 1               5                   10                  15
Val Arg Phe Glu
            20
```

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```
Val Leu Pro Ala Leu Pro Gln Val Val
 1               5
```

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
Cys Val Ala Gln Pro Gly Pro Gln Val Leu Leu Val Leu Cys
 1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Cys Val Ala Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys
1 5 10 15

WHAT IS CLAIMED IS:

1. An isolated protein, the amino acid sequence of which consists of amino acid numbers selected from the group consisting of 41-54, 45-54, 47-53, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-56, 47-58, 58-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-5, 8-19, 21-23 and 33-35, respectively) as depicted in Figure 8 (a portion of SEQ ID NO:2).

2. The isolated protein of claim 1 which is N-acetylated or has a C-terminal amide or is both N-acetylated and has a C-terminal amide.

3. An isolated derivative of a protein of claim 1, which derivative has activity against HIV infection or replication.

4. The isolated derivative of claim 3, which contains an insertion of or substitution with one or more non-classical amino acids or one or more D-amino acids.

5. An isolated protein, the amino acid sequence of which consists of two or more at least five amino acid, non-naturally contiguous portions of the sequence of β-hCG as depicted in Figure 8 (SEQ ID NO:2) in which the portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion, said protein being active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-hematopoietic activity.

6. The isolated protein of claim 5 in which the amino acid sequence of said protein is selected from the group consisting of β-hCG amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 109-119 (SEQ ID NO:7), β-hCG amino acids 110-119

(SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 45-57 (SEQ ID NO:6), and β-hCG amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 108-119 (SEQ ID NO:29), as depicted in Figure 8 (SEQ ID NO:2).

7. An isolated protein, the amino acid sequence of which comprises two or more at least five amino acid portions of the sequence of β-hCG as depicted in Figure 8 (SEQ ID NO:2) said protein being active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-hematopoietic activity.

8. The isolated protein of claim 7 in which said portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion.

9. The isolated protein of claim 7 which is a fusion protein comprising the β-hCG amino acid sequences joined via a peptide bond to a protein sequence of a protein different from β-hCG.

10. A circularized protein, the amino acid sequence of which consists of one or more portions of the sequence of β-hCG as depicted in Figure 8 (SEQ ID NO:2) in which a cysteine residue is inserted or substituted for a different amino acid residue in at least one of said portions of said sequence, said one or more portions of said sequence containing a second cysteine residue, and in which a disulfide bond is formed between the inserted or substituted cysteine residue and the second cysteine residue present in said one or more portions of said sequence, said protein being active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-h matopoietic activity.

11. The circularized protein of claim 10 in which said at least one portion of said sequence is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-57, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively), as depicted in Figure 8 (a portion of SEQ ID NO:2).

12. The circularized protein of claim 10, the amino acid sequence of which consists of β-hCG amino acid numbers 44-57 (SEQ ID NO:12), as depicted in Figure 8 (a portion of SEQ ID NO:2), with cysteine substituted for valine at position 44.

13. The circularized protein of claim 10, the amino acid sequence of which consists of two or more at least 5 amino acid, non-naturally contiguous portions of the sequence of β-hCG as depicted in Figure 8 (SEQ ID NO:2) in which the portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion.

14. The circularized protein of claim 13, in which the first said portion consists of β-hCG amino acid numbers 45-57 (SEQ ID NO:6) and the second said portion consists of β-hCG amino acid numbers 110-119 (SEQ ID NO:27); and in which a disulfide bond is formed between the cysteine residues at amino acids 57 and 110 of said portions.

15. An isolated protein, the amino acid sequence of which consists of one or more portions of the sequence of β-hCG as depicted in Figure 8 (SEQ ID NO:2) in which one or more residues in at least one of said portions of said sequence are substituted by an amino acid or amino acid analog having a side chain with an amino or carboxyl group, said amino or carboxyl group forming a peptide bond with a

second sequence of one or more amino acids, said protein being active to inhibit HIV infection or replication or Kaposi's Sarcoma or having a pro-hematopoietic activity.

16. The isolated protein of claim 15, in which said at least one portion of said sequence consists of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-57, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively), as depicted in Figure 8 (a portion of SEQ ID NO:2).

17. The isolated protein of claim 16, in which said at least one portion of said sequence consists of amino acid numbers 45-57 (SEQ ID NO:6) as depicted in Figure 8 (a portion of SEQ ID NO:2), and in which substitutions by said amino acid or amino acid analog occur at least at residues 47 and 51 of said portion.

18. The isolated protein of claim 15, in which the one or more residues are each substituted by a diaminobutyric acid residue and the side chain amino group of said diaminobutyric acid residue is peptide bonded to a sequence of one or more proline residues.

19. An isolated protein, the amino acid sequence of which consists of amino acid numbers 45-57 of the sequence of β-hCG as depicted in Figure 8 (a portion of SEQ ID NO:2) in which the residues at positions 47 and 51 are each substituted by a diaminobutyric acid residue and the side chain amino group of said diaminobutyric acid residue is peptide bonded to a proline residue, said protein being active to inhibit HIV infection or replication or Kaposi's Sarcoma or having a pro-hematopoietic activity.

20. A circularized protein, the amino acid sequence of which consists of one or more portions of the sequence of β-hCG as depicted in Figure 8 (SEQ ID NO:2) in which a cysteine residue is inserted or substituted for a different amino acid residue in at least one of said one or more portions of said sequence, said one or more portions of said sequence containing a second cysteine residue, and in which a disulfide bond is formed between the inserted or substituted cysteine residue and the second cysteine residue present in said one or more portions of said sequence, and also in which one or more residues said at least one or said one or more portions of said sequence are substituted by an amino acid or amino acid analog having a side chain with an amino or carboxyl group, said amino or carboxyl group forming a peptide bond with a second sequence of one or more amino acids, said protein being active to inhibit HIV infection or replication or Kaposi's Sarcoma or having a pro-hematopoietic activity.

21. A circularized protein, the amino acid sequence of which consists of β-hCG amino acid numbers 44-57 (SEQ ID NO:12) as depicted in Figure 8 (a portion of (SEQ ID NO:2) with cysteine substituted for valine at position 44, and in which a disulfide bond is formed between the cysteine residue substituted at position 44 and the cysteine residue present at position 57, and in which the residues at positions 47 and 51 are each substituted by a diaminobutyric acid residue and the side chain amino group of said diaminobutyric acid residue is peptide bonded to a proline residue.

22. An isolated protein (a) comprising a β-hCG amino acid sequence consisting of amino acid numbers 41-54, 45-54, 47-53, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-56, 47-58, 58-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-5, 8-19, 21-23 and 33-35, respectively)as depicted in Figure 8 (a portion of SEQ ID

NO:2); and (b) lacking β-hCG amino acids contiguous to said sequence.

23. The isolated protein of claim 22 which is a fusion protein comprising the β-hCG amino acid sequence joined via a peptide bond to a protein sequence of a protein different from β-hCG.

24. The protein of claim 23 in which the β-hCG amino acid sequence is joined via a peptide bond to a protein sequence of a chemokine.

25. The protein of claim 24 in which the chemokine is selected from the group consisting of RANTES, MIP-1α and MIP-1β.

26. The protein of claim 22 which is circularized.

27. A derivative of the protein of claim 22, containing one or more conservative substitutions in said sequence.

28. A first composition comprising one or more first components of a second composition comprising a sample of native hCG or native β-hCG, said first components being separated from other components of the hCG or β-hCG sample, said first components being active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-hematopoietic activity, and said second composition being active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-hematopoietic activity, and said native hCG or β-hCG not being purified to homogeneity in said second composition.

29. The first composition of claim 28 in which said first components are separated from said other components by sizing column chromatography.

30. The first composition of claim 29 in which said sizing column chromatography is performed using a SUPERDEX™ 200 column.

31. The first composition of claim 28 in which said first components have an approximate apparent molecular weight selected from the group consisting of 40 kD, 15 kD and 3 kD, wherein said apparent molecular weight is determined by elution from a gel filtration sizing column relative to the elution of a native hCG heterodimer, having a molecular weight of 77 kD, and a β-hCG core protein, having a molecular weight of 10 kD.

32. A first composition produced by a process comprising the following steps:

(a) subjecting a second composition comprising native hCG or native β-hCG, said second composition being active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-hematopoietic activity, said native hCG or native β-hCG not being purified to homogeneity in said second composition, to a size fractionation procedure; and

(b) recovering fractions active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-hematopoietic activity.

33. The first composition of claim 32 in which the recovered fractions contain material having an approximate apparent molecular weight selected from the group consisting of 40 kD, 15 kD and 3 kD, wherein said apparent molecular weight is determined by elution from a gel filtration sizing column relative to the elution of a native hCG heterodimer, having a molecular weight of 77 kD, and a β-hCG core protein, having a molecular weight of 10 kD.

34. The first composition of claim 32, in which said second composition is early pregnancy urine.

35. A method for producing a first composition active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-hematopoietic activity, comprising

(a) subjecting a second composition comprising native hCG or native β-hCG, said second composition being active to inhibit HIV infection or replication of Kaposi's sarcoma or having a pro-hematopoietic activity, said native hCG or native β-hCG not being purified to homogeneity in said second composition, to a size fractionation procedure; and

(b) recovering fractions active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-hematopoietic activity.

36. The method of claim 35 in which the recovered fractions contain material having an approximate apparent molecular weight selected from the group consisting of 40 kD, 15 kD and 3 kD, wherein said apparent molecular weight is determined by elution from a gel filtration sizing column relative to the elution of a native hCG heterodimer, having a molecular weight of 77 kD, and a β-hCG core protein, having a molecular weight of 10 kD.

37. The method of claim 35 in which the size fractionation procedure comprises the steps

(a) loading the second composition onto a gel filtration sizing column in a first buffer of 30 mM sodium phosphate, pH 8.3;

(b) eluting components of the second composition from the column with second buffer of 30 mM sodium phosphate, pH 7.0 and 2 M sodium chloride; and

(c) recovering fractions of the second composition having been eluted from the column.

38. The method of claim 37 in which the gel filtration sizing column is a SUPERDEX 200™ column and in which the recovered fractions contain material having an approximate

apparent molecular weight s lected from the group consisting of 40 kD, 15 kD and 3 kD, wherein said apparent molecular weight is determined by elution from the SUPERDEX™ 200 column relative to the elution of a native hCG heterodimer, having a molecular weight of 77 kD, and a β-hCG core protein, having a molecular weight of 10 kD.

39. The method of claim 37 in which the second composition is early pregnancy urine.

40. The method of claim 39 in which prior to subjecting the second composition to a size fractionation procedure, the second composition is subjected to the following steps:

(a) adjusting the pH of the urine to a pH of approximately 7.2 causing the formation of a precipitate;

(b) removing the precipitate from the urine;

(c) concentrating the urine;

(d) removing salt and lipid from the urine; and

(e) lyophilizing the urine.

41. A method of treating or preventing HIV infection in a human subject in need of such treatment or prevention comprising administering to the subject an amount of a purified protein effective to treat or prevent HIV infection in which the protein comprises one or more portions of the amino acid sequence of β-hCG, a peptide having an amino acid sequence consisting of said one or more portion being active to inhibit HIV infection or replication.

42. The method of claim 41 in which the amino acid sequence of at least one of said one or more portions is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 7-40, 46-65 and 48-56 (SEQ ID

NOS:3-6, 8-24, and 33-35, respectively), as depicted in Figure 8 (a portion of SEQ ID NO:2).

43. The method of claim 41 in which the purified protein comprises two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 8 (SEQ ID NO:2), in which said portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion.

44. The method of claim 43, in which the amino acid sequence of said protein is selected from the group consisting of β-hCG amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 109-119 (SEQ ID NO:7), β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 45-57 (SEQ ID NO:6), and β-hCG amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 108-119 (SEQ ID NO:29), as depicted in Figure 8 (SEQ ID NO:2).

45. The method of claim 41 which further comprises administering to the subject a chemokine or an anti-viral drug other than β-hCG.

46. The method of claim 41 in which the protein is administered intramuscularly.

47. The method of claim 41 in which the protein is N-acetylated or has a C-terminal amide or is both N-acetylated and has a C-terminal amide.

48. A method of treating or preventing HIV infection in a human subject in need of such treatment or prevention comprising administering to the subject an amount of a

purified derivativ of a protein effective to tr at or prevent HIV infection, in which the protein compris s one or more portions of the amino acid sequence of β-hCG, a peptide having an amino acid sequence consisting of said one or more portions being active to inhibit HIV infection or replication.

49. The method of claim 48 in which the amino acid sequence of at least one of said one or more portions is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25, and 33-35 respectively), as depicted in Figure 8 (a portion of SEQ ID NO:2).

50. The method of claim 48 in which the purified derivative comprises two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 8 (SEQ ID NO:2), in which said portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion.

51. The method of claim 50, in which the amino acid sequence of said derivative is selected from the group consisting of β-hCG amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 109-119 (SEQ ID NO:7), β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 45-57 (SEQ ID NO:6), and β-hCG amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 108-119 (SEQ ID NO:29) as depicted in Figure 8 (SEQ ID NO:2).

52. The method of claim 48 which further comprises administering to the subject a chemokine or an anti-viral drug other than β-hCG.

53. The method of claim 48 in which the protein is administered intramuscularly.

54. The method of claim 48 in which the protein is N-acetylated or has a C-terminal amide or is both N-acetylated and has a C-terminal amide.

55. A method of treating or preventing HIV infection in a human subject in need of such treatment or prevention comprising administering to the subject an amount of a circularized protein effective to treat HIV infection, the amino acid sequence of which protein consists of one or more portions of the sequence of β-hCG as depicted in Figure 8 (a portion of SEQ ID NO:2), in which a cysteine residue is inserted or substituted for a different amino acid residue in at least one of said one or more portions of said sequence, said one or more portions of said sequence containing a second cysteine residue, and in which a disulfide bond is formed between the inserted or substituted cysteine residue and the second cysteine residue present in said one or more portions of said sequence, said circularized protein being active to inhibit HIV infection or replication.

56. The method of claim 55, in which the amino acid sequence of said at least one portion is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 45-57, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-57, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, and 48-56 (SEQ ID NOS:3-25 and 33-35 respectively), as depicted in Figure 8 (a portion of SEQ ID NO:2).

57. The method of claim 56, in which the circularized protein has an amino acid sequence which consists of β-hCG amino acid numbers 44-57 (SEQ ID NO:12), as depicted in Figure 8 (a portion of SEQ ID NO:2), with cysteine substituted for valine at position 44.

58. The method of claim 55 in which said circularized protein consists of two or more at least five amino acid, non-naturally contiguous portions of the sequence of β-hCG as depicted in Figure 8 (SEQ ID NO:2), in which the portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion.

59. The method of claim 58, in which the first portion consists of β-hCG amino acid numbers 45-57 (SEQ ID NO:6) and the second portion consists of β-hCG amino acid numbers 110-119 (SEQ ID NO:27) as depicted in Figure 8 (SEQ ID NO:2); and in which a disulfide bond is formed between the cysteine residues at a amino acids 57 and 110 of said portions.

60. A method of treating or preventing HIV infection in a human subject in need of such treatment or prevention comprising administering to the subject an amount of a purified protein effective to treat or prevent HIV infection, in which the protein has an amino acid sequence which consists of one or more portions of the sequence of β-hCG as depicted in Figure 8 (SEQ ID NO:2) in which one or more residues in at least one of said one or more portion of said sequence are substituted by an amino acid or amino acid analog having a side chain with an amino or carboxyl group, said amino or carboxyl group forming a peptide bond with a second sequence of one or more amino acids, said protein being active to inhibit HIV infection or replication.

61. The method of claim 60, in which said at least one portion of said sequence is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57,

109-119, 45-57, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-57, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, and 48-56 (SEQ ID NOS:3-25 and 33-35 respectively), as depicted in Figure 8 (a portion of SEQ ID NO:2).

62. The method of claim 61, in which said at least one portion of said sequence consists of amino acid numbers 45-57 (SEQ ID NO:6) as depicted in Figure 8 (a portion of SEQ ID NO:2) with substitutions by said amino acid or amino acid analog at residues 47 and 51 of said portion.

63. The method of claim 60, in which the one or more residues are each substituted by a diaminobutyric acid residue and the side chain amino group of said diaminobutyric acid residue is peptide bonded to a sequence of one or more proline residues.

64. The method of claim 62, in which the residues at positions 47 and 51 of said portion are each substituted by a diaminobutyric acid residue and the side chain amino group of said diaminobutyric acid residue is peptide bonded to a proline residue.

65. A method of treating or preventing HIV infection in a human subject in need of such treatment or prevention comprising administering to the subject an amount of a circularized protein effective to treat HIV infection, the amino acid sequence of which protein consists of one or more portions of the sequence of β-hCG as depicted in Figure 8 (a portion of SEQ ID NO:2) in which a cysteine residue is inserted or substituted for a different amino acid residue in at least one of said one or more portions of said sequence, said one or more portions of said sequence containing a second cysteine residue, and in which a disulfide bond is formed betwe n the inserted or substituted cysteine residue and the second cysteine residue present in said one or more

portions of said sequence, and also in which one or more residues in at least one of said one or more portions of said sequence are substituted by an amino acid or amino acid analog having a side chain with an amino or carboxyl group, said amino or carboxyl group forming a peptide bond with a second sequence of one or more amino acids, said circularized protein being active to inhibit HIV infection or replication.

66. The method of claim 65, in which said circularized protein has an amino acid sequence which consists of β-hCG amino acid numbers 44-57 (SEQ ID NO:12) as depicted in Figure 8 (a portion of (SEQ ID NO:2) with cysteine substituted for valine at position 44, and in which the residues at positions 47 and 51 of said portion are each substituted by a diaminobutyric acid residue and the side chain amino group of said diaminobutyric acid residue is peptide bonded to a proline residue.

67. A method of treating or preventing HIV infection in a human subject in need of such treatment or prevention comprising administering to the subject an amount of a purified protein effective to treat or prevent HIV infection, which protein (a) comprises a portions of the amino acid sequence of β-hCG, a peptide having an amino acid sequence consisting of said portion being active to inhibit HIV infection or replication; and (b) lacks β-hCG amino acids contiguous to said portion.

68. The method of claim 68 in which the portion has a sequence selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 45-57, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-57, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, and 48-56 (SEQ ID NOS:3-25 and 33-35 respectively), as depicted in Figure 8 (a portion of SEQ ID NO:2).

69. The method of claim 67 in which the portion consists of amino acid numbers 45-57 (SEQ ID NO:6) as depicted in Figure 8 (a portion of SEQ ID NO:2).

70. A method of treating or preventing HIV infection in a human subject in need of such treatment or prevention comprising administering to the subject an amount of the first composition of claim 28 effective to treat or prevent HIV infection.

71. The method of claim 70 in which said first components have an approximate apparent molecular weight selected from the group consisting of 40 kD, 15 kD and 3 kD, wherein said apparent molecular weight is determined by elution from a gel filtration sizing column relative to the elution of a native hCG heterodimer, having a molecular weight of 77 kD, and a β-hCG core protein, having a molecular weight of 10 kD.

72. A method of treating or preventing HIV infection in a human subject in need of such treatment or prevention comprising administering to the subject an amount of a first composition effective to treat or prevent HIV infection, said first composition being produced by a process comprising the following steps:

(a) subjecting a second composition comprising native hCG or native β-hCG, said second composition being active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-hematopoietic activity, said native hCG or native β-hCG not being purified to homogeneity in said second composition, to a size fractionation procedure; and

(b) recovering fractions active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-hematopoietic activity.

73. The method of claim 72, in which the recovered fractions contain material having an approximate apparent molecular weight selected from the group consisting of 40 kD, 15 kD and 3 kD, wherein said apparent molecular weight is determined by elution from a gel filtration sizing column relative to the elution of a native hCG heterodimer, having a molecular weight of 77 kD, and a β-hCG core protein, having a molecular weight of 10 kD.

74. A method of screening a preparation comprising hCG or β-hCG or a fraction of an hCG or β-hCG preparation or one or more portions of β-hCG for anti-HIV activity comprising assaying said fraction for the ability to inhibit HIV replication or expression of HIV RNA or protein.

75. The method of claim 74 in which the preparation is assayed by a method comprising measuring HIV-1 p24 antigen levels in cultured hematopoietic cells acutely infected with HIV-1, which cells have been contacted with the fraction; and comparing the measured HIV-1 p24 antigen levels in the cells which have been contacted with the fraction with said levels in cells not so contacted with the fraction, wherein a lower level in said contacted cells indicates that the fraction has anti-HIV activity.

76. The method of claim 74 in which the preparation is assayed by a method comprising measuring the activity of a reporter gene product expressed from a construct in which the HIV-1 LTR is operably linked to said reporter gene, wherein said construct is present in cultured cells which have been contacted with the fraction; and comparing the measured expression of said reporter gene in the cells which have been contacted with the fraction with said levels in such cells not so contacted, wherein a lower level in said contacted cells indicates that the fraction has anti-HIV activity.

77. The method of claim 74 in which the preparation is assayed by a method comprising measuring HIV-1 derived RNA transcripts or HIV-1 antigen levels in HIV-1 transgenic mice administered the fraction; and comparing the measured transcript or antigen levels in the mice which have been administered the fraction with said levels in mice not so administered, wherein a lower level in said administered mice indicates that the fraction has anti-HIV activity.

78. The method of claim 74 in which the preparation is assayed by a method comprising measuring SIV p27 antigen levels in the peripheral blood mononuclear cells of SIV infected monkeys administered the fraction; and comparing the measured antigen levels in the monkeys which have been administered the fraction with said levels in monkeys not so administered, wherein a lower level in said administered monkeys indicates that the fraction has anti-HIV activity.

79. A pharmaceutical composition comprising a therapeutically effective amount of a purified protein, the amino acid sequence of which protein is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-56, 47-58, 48-145, 58-145, 7-40, 46-65 and 58-56 (SEQ ID NOS:3-5, 8-19, 21, 22, 24, and 33-35, respectively) as depicted in Figure 8 (a portion of SEQ ID NO:2); and a pharmaceutically acceptable carrier.

80. A pharmaceutical composition comprising a therapeutically effective amount of a purified protein effective to treat or prevent HIV, the amino acid sequence of which protein comprises two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 8 (SEQ ID NO:2), in which the portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion; and a pharmaceutically acceptable carrier.

81. The pharmaceutical composition of claim 79 or 80 which is formulated as a controlled release formulation.

82. The pharmaceutical composition of claim 79 or 80 which further comprises a therapeutically effective amount of a chemokine.

83. The pharmaceutical composition of claim 80, in which the amino acid sequence of said protein is selected from the group consisting of β-hCG amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 109-119 (SEQ ID NO:7), β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 45-57 (SEQ ID NO:6), and β-hCG amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 108-119 (SEQ ID NO:29) as depicted in Figure 8 (SEQ ID NO:2).

84. The pharmaceutical composition of claim 80 in which the protein is a fusion protein, said fusion protein comprising at least one of said portions of the β-hCG amino acid sequence joined via a peptide bond to a sequence of a protein different from β-hCG.

85. A pharmaceutical composition comprising a therapeutically effective amount of a circularized protein, the amino acid sequence of which consists of one or more portions of the sequence of β-hCG as depicted in Figure 8 (a portion of SEQ ID NO:2) in which a cysteine residue is inserted or substituted for a different amino acid residue in at least one of said one or more portions of said sequence, said one or more portions of said sequence containing a second cysteine residue, and in which a disulfide bond is formed between the inserted or substituted cysteine residue and the second cysteine residue present in said one or more portions of said sequence, said circularized protein being

active to inhibit HIV infection or replication; and a pharmaceutically acceptable carrier.

86. The pharmaceutical composition of claim 85 in which the sequence of said at least one portion is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-57, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively), as depicted in Figure 8 (a portion of SEQ ID NO:2).

87. The pharmaceutical composition of claim 86, in which the circularized protein has an amino acid sequence which consists of β-hCG amino acid numbers 44-57 (SEQ ID NO:12), as depicted in Figure 8 (a portion of SEQ ID NO:2), with cysteine substituted for valine at position 44.

88. The pharmaceutical composition of claim 85, in which the amino acid sequence of said circularized protein consists of two or more at least five amino acid, non-naturally contiguous portions of the β-hCG sequence as depicted in Figure 8 (SEQ ID NO:2) in which the portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion.

89. The pharmaceutical composition of claim 88, in which the first portion consists of β-hCG amino acids 45-57 (SEQ ID NO:6) and the second portion consists of β-hCG amino acids 110-119 (SEQ ID NO:27) as depicted in Figure 8 (SEQ ID NO:2); and in which a disulfide bond is formed between the cysteine residue at amino acids 57 and 110 of said portions.

90. A pharmaceutical composition comprising a therapeutically effective amount of a purified protein, the amino acid sequence of which protein consists of one or more portions of the sequence of β-hCG as depicted in Figure 8

(SEQ ID NO:2), in which one or more residues in at least one of said one or more portions are substituted by an amino acid or amino acid analog having a side chain with an amino or carboxyl group, said amino or carboxyl group forming a peptide bond with a second sequence of one or more amino acids, said purified protein being active to inhibit HIV infection or replication; and a pharmaceutically acceptable carrier.

91. The pharmaceutical composition of claim 90, in which said at least one portion of said sequence has a sequence selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-57, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively), as depicted in Figure 8 (a portion of SEQ ID NO:2).

92. The pharmaceutical composition of claim 90, in which said at least one portion of said sequence consists of amino acid numbers 45-57 (SEQ ID NO:6) as depicted in Figure 8 (a portion of SEQ ID NO:2), and in which substitutions by said amino acid or amino acid analog occur at residues 47 and 51 of said portion.

93. The pharmaceutical composition of claim 90, in which said one or more residues are each substituted by a diaminobutyric acid residue and the side chain amino group of said diaminobutyric acid residue is peptide bonded to a sequence of one or more proline residues.

94. The pharmaceutical composition of claim 92, in which the residues at positions 47 and 51 of said at least one portion are each substituted by a diaminobutyric acid residue and the side chain amino group of said diaminobutyric acid residue is peptide bonded to a proline residue.

95. A pharmaceutical composition comprising a therapeutically effective amount of a circularized protein, the amino acid sequence of which consists of one or more portions of the sequence of β-hCG as depicted in Figure 8 (a portion of SEQ ID NO:2), in which a cysteine residue is inserted or substituted for a different amino acid residue in at least one of said one or more portions of said sequence, said one or more portions of said sequence containing a second cysteine residue, and in which a disulfide bond is formed between the inserted or substituted cysteine residue and the second cysteine residue present in said one or more portions of said sequence, and also in which one or more residues in at least one of said one or more portions of said sequence are substituted by an amino acid or amino acid analog having a side chain with an amino or carboxyl group, said amino or carboxyl group forming a peptide bond with a second sequence of one or more amino acids, said circularized protein being active to inhibit HIV infection or replication; and a pharmaceutically acceptable carrier.

96. The pharmaceutical composition of claim 95, in which said at least one portion of said sequence consists of β-hCG amino acid numbers 44-57 (SEQ ID NO:12) as depicted in Figure 8 (a portion of (SEQ ID NO:2), and in which cysteine is substituted for valine at position 44, and in which the residues at positions 47 and 51 of said portion are each substituted by a diaminobutyric acid residue and the side chain amino group of said diaminobutyric acid residue is peptide bonded to a proline residue.

97. A pharmaceutical composition comprising a therapeutically effective amount of a purified nucleic acid encoding a protein, the amino acid sequence of which protein is selected from the group consisting of 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-

25 and 33-35, respectively), as depicted in Figure 8 (a portion of SEQ ID NO. 2); and a pharmaceutically acceptable carrier.

98. A pharmaceutical composition comprising a therapeutically effective amount of a purified nucleic acid encoding a protein, the amino acid sequence of which protein comprises two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 8 (SEQ ID NO:2), in which the portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion; and a pharmaceutically acceptable carrier.

99. The pharmaceutical composition of claim 99, in which the amino acid sequence of said protein is selected from the group consisting of β-hCG amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 109-119 (SEQ ID NO:7), β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 45-57 (SEQ ID NO:6), and β-hCG amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 108-119 (SEQ ID NO:29) as depicted in Figure 8 (SEQ ID NO:2).

100. The pharmaceutical composition of claim 97 or 98 in which the nucleic acid is a nucleic acid vector.

101. A pharmaceutical composition comprising a recombinant cell containing a nucleic acid vector encoding a protein, the amino acid sequence of which protein is selected from the group consisting of 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25 and 33-

35, resp ctively), as depicted in Figure 8 (a portion of SEQ ID NO. 2); and a pharmaceutically acceptable carrier.

102. The pharmaceutical composition of claim 101 in which the cell is a hematopoietic cell.

103. A pharmaceutical composition comprising a recombinant cell containing a nucleic acid vector encoding a protein comprising two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 8 (SEQ ID NO:2), in which the portions are linked via a peptide bond between the N-terminus of a first said portion and C-terminus of a second said portion; and a pharmaceutically acceptable carrier.

104. A pharmaceutical composition comprising (a) a purified protein, which protein (i) comprises a β-hCG amino acid sequence selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-57, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively), as depicted in Figure 8 (a portion of SEQ ID NO:2), and (ii) lacks β-hCG amino acids contiguous to said sequence; and a pharmaceutically acceptable carrier.

105. The pharmaceutical composition of claim 104 in which the β-hCG amino acid sequence consists of amino acid numbers 45-57 (SEQ ID NO:6) as depicted in Figure 8 (a portion of SEQ ID NO:2).

106. The pharmaceutical composition of claim 104 in which the protein is a fusion protein, said fusion protein comprising said β-hCG amino acid sequence joined via a peptide bond to a sequence of a protein different from β-hCG.

107. The pharmaceutical composition of claim 106 in which said amino acid sequence is joined via a peptide bond to a sequence of a chemokine.

108. A pharmaceutical composition comprising (a) a therapeutically effective amount of a purified nucleic acid encoding a protein, which protein (i) comprises a β-hCG amino acid sequence selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-57, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively), as depicted in Figure 8 (a portion of SEQ ID NO:2), and (ii) lacks β-hCG amino acids contiguous to said sequence; and (b) a pharmaceutically acceptable carrier.

109. The pharmaceutical composition of claim 108 in which the nucleic acid is a nucleic acid vector.

110. A pharmaceutical composition comprising (a) a recombinant cell containing a nucleic acid vector encoding a protein, which protein (i) comprises a β-hCG amino acid sequence selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-57, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively) as depicted in Figure 8 (a portion of SEQ ID NO:2), and (ii) lacks β-hCG amino acids contiguous to said sequence; and (b) a pharmaceutically acceptable carrier.

111. The pharmaceutical composition of claim 110 in which the nucleic acid vector encodes a protein, the amino acid sequence of which consists of amino acid numbers 45-57 (SEQ ID NO:6) as depicted in Figure 8 (a portion of SEQ ID NO:2).

112. A pharmaceutical composition comprising a therapeutically effective amount of a derivativ of a protein, the amino acid sequence of which protein is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively), as depicted in Figure 8 (a portion of SEQ ID NO. 2), said derivative being active to inhibit HIV infection of replication; and a pharmaceutically acceptable carrier.

113. A pharmaceutical composition comprising a therapeutically effective amount of a derivative of a protein, the amino acid sequence of which protein comprises two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 8 (SEQ ID NO:2), in which the portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion, said derivative being active to inhibit HIV infection or replication; and a pharmaceutically acceptable carrier.

114. The pharmaceutical composition of claim 113, in which the amino acid sequence of said protein is selected from the group consisting of β-hCG amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 109-119 (SEQ ID NO:7), β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 45-57 (SEQ ID NO:6), and β-hCG amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 108-119 (SEQ ID NO:29), as depicted in Figure 8 (SEQ ID NO:2).

115. The pharmaceutical composition of claim 112 or 113 in which the derivative contains one or more D-amino acids or non-classical amino acids.

116. The pharmaceutical composition of claim 112 or 113 which is formulated as a controlled release formulation.

117. The pharmaceutical composition of claim 112 or 113 which further comprises a therapeutically effective amount of a chemokine.

118. A pharmaceutical composition comprising a therapeutically effective amount of a purified nucleic acid encoding a derivative of a protein, the amino acid sequence of which protein is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively), as depicted in Figure 8 (a portion of SEQ ID NO. 2), said derivative being active to inhibit HIV infection of replication; and a pharmaceutically acceptable carrier.

119. The pharmaceutical composition of claim 118 in which the nucleic acid is a nucleic acid vector.

120. A pharmaceutical composition comprising a recombinant cell containing a nucleic acid vector encoding a derivative of a protein, the amino acid sequence of which protein is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively), as depicted in Figure 8 (a portion of SEQ ID NO. 2), said derivative being active to inhibit HIV infection of replication; and a pharmaceutically acceptable carrier.

121. A pharmaceutical composition comprising a therapeutically eff ctive amount of a purified nucleic acid encoding a derivative of a protein, the amino acid sequence of which protein comprises two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 8 ((SEQ ID NO:2), in which the portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion, said derivative being active to inhibit HIV infection or replication; and a pharmaceutically acceptable carrier.

122. The pharmaceutical composition of claim 121 in which the nucleic acid is a nucleic acid vector.

123. A pharmaceutical composition comprising a recombinant cell containing a nucleic acid vector encoding a derivative of a protein, the amino acid sequence of which protein comprises two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 8 (SEQ ID NO:2), in which the portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion, said derivative being active to inhibit HIV infection or replication; and a pharmaceutically acceptable carrier.

124. A pharmaceutical composition comprising a therapeutically effective amount of a first composition comprising one or more first components of a second composition comprising a sample of native hCG or native β-hCG, said first components being separated from other components of the hCG or β-hCG sample, said first components being active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-hematopoietic activity, and said second composition being active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-

hematopoietic activity, and said nativ hCG or β-hCG n t being purified to homogeneity in said second composition; and a pharmaceutically acc ptabl carrier.

125. The pharmaceutical composition of claim 124 in which said first components are separated from said other components by gel filtration sizing column chromatography.

126. The pharmaceutical composition of claim 125 in which said sizing column chromatography is performed using a SUPERDEX™ 200 column.

127. The pharmaceutical composition of claim 125 in which said first components have an approximate apparent molecular weight selected from the group consisting of 40 kD, 15 kD and 3 kD, wherein said apparent molecular weight is determined by elution from a gel filtration sizing column relative to the elution of a native hCG heterodimer, having a molecular weight of 77 kD, and a β-hCG core protein, having a molecular weight of 10 kD.

128. A pharmceutical composition comprising a therapeutically effective amount of a first composition effective to treat or prevent HIV infection, said first composition produced by a process comprising the following steps:

(a) subjecting a second composition comprising native hCG or native β-hCG, said second composition being active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-hematopoietic activity, said native hCG or native β-hCG not being purified to homogeneity in said second composition, to a size fractionation procedure; and

(b) recovering fractions active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-hematopoietic activity;

and a pharmaceutically acceptable carrier.

129. The pharmaceutical composition of claim 128, in which the recovered fractions contain material having an approximate apparent molecular weight selected from the group consisting of 40 kD, 15 kD and 3 kD, wherein said apparent molecular weight is determined by elution from a gel filtration sizing column relative to the elution of a native hCG heterodimer, having a molecular weight of 77 kD, and a β-hCG core protein, having a molecular weight of 10 kD.

130. The pharmaceutical composition of claim 128, in which the sample of hCG is early pregnancy urine.

131. A method for producing a protein comprising chemically synthesizing a protein, the amino acid sequence of which protein comprises two or more at least 5 amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG, said portions being linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion, said protein being active to inhibit HIV infection or replication or Kaposi's Sarcoma or having a pro-hematopoietic activity.

132. A method of treating or preventing HIV infection in a human subject in need of such treatment or prevention comprising administering to the subject an amount, effective to treat or prevent HIV infection, of a purified nucleic acid encoding a protein comprising one or more portions of the amino acid sequence of β-hCG, a peptide having an amino acid sequence consisting of said one or more portions being active to inhibit HIV infection or replication.

133. A method of treating or preventing HIV infection in a human subject in need of such treatment or prevention comprising administering to the subject an amount, effective to treat or prevent HIV infection, of a purified nucleic acid encoding a derivative of a protein in which the protein comprises on or more portions of the amino acid sequence of

β-hCG, a peptide having an amino acid sequence consisting of said one or more portions being active to inhibit HIV infection or replication.

134. A method of treating or preventing HIV infection in a human subject in need of such treatment or prevention comprising administering to the subject an amount, effective to treat or prevent HIV infection, of a nucleic acid encoding a protein, the amino acid sequence of which protein comprises two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 8 (SEQ ID NO:2), in which the portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion, a peptide having an amino acid sequence comprising said portions being active to inhibit HIV infection or replication.

135. A method of treating or preventing HIV infection in a human subject in need of such treatment or prevention comprising administering to the subject an amount, effective to treat or prevent HIV infection, of a recombinant cell containing a nucleic acid encoding a protein, the amino acid sequence of which comprises two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 8 ((SEQ ID NO:2), in which the portions are linked via a peptide bond at the N-terminus of a first said portion and the C-terminus of a second said portion, a peptide having an amino acid sequence comprising said portions being active to inhibit HIV infection or replication.

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Figures 1A-D

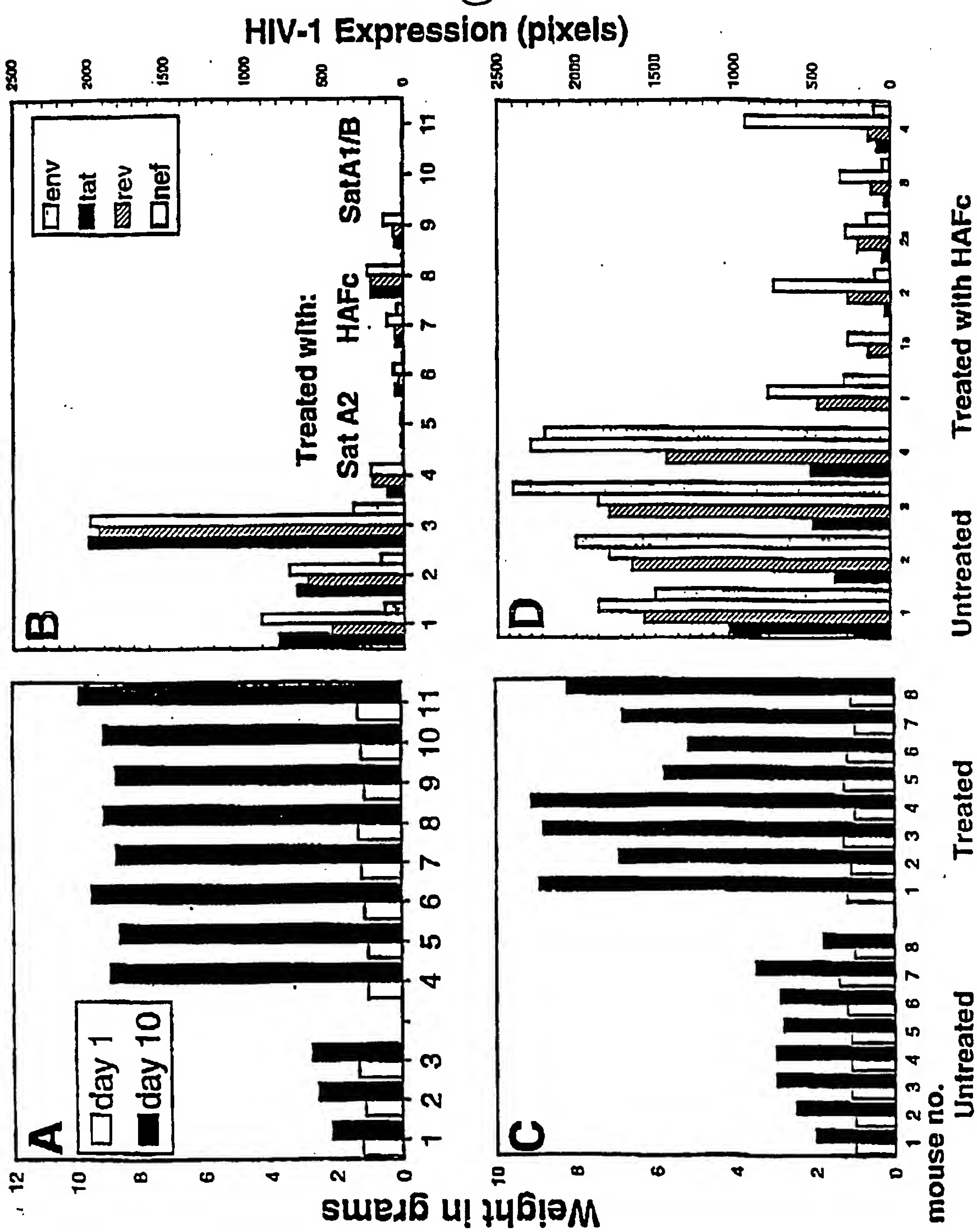

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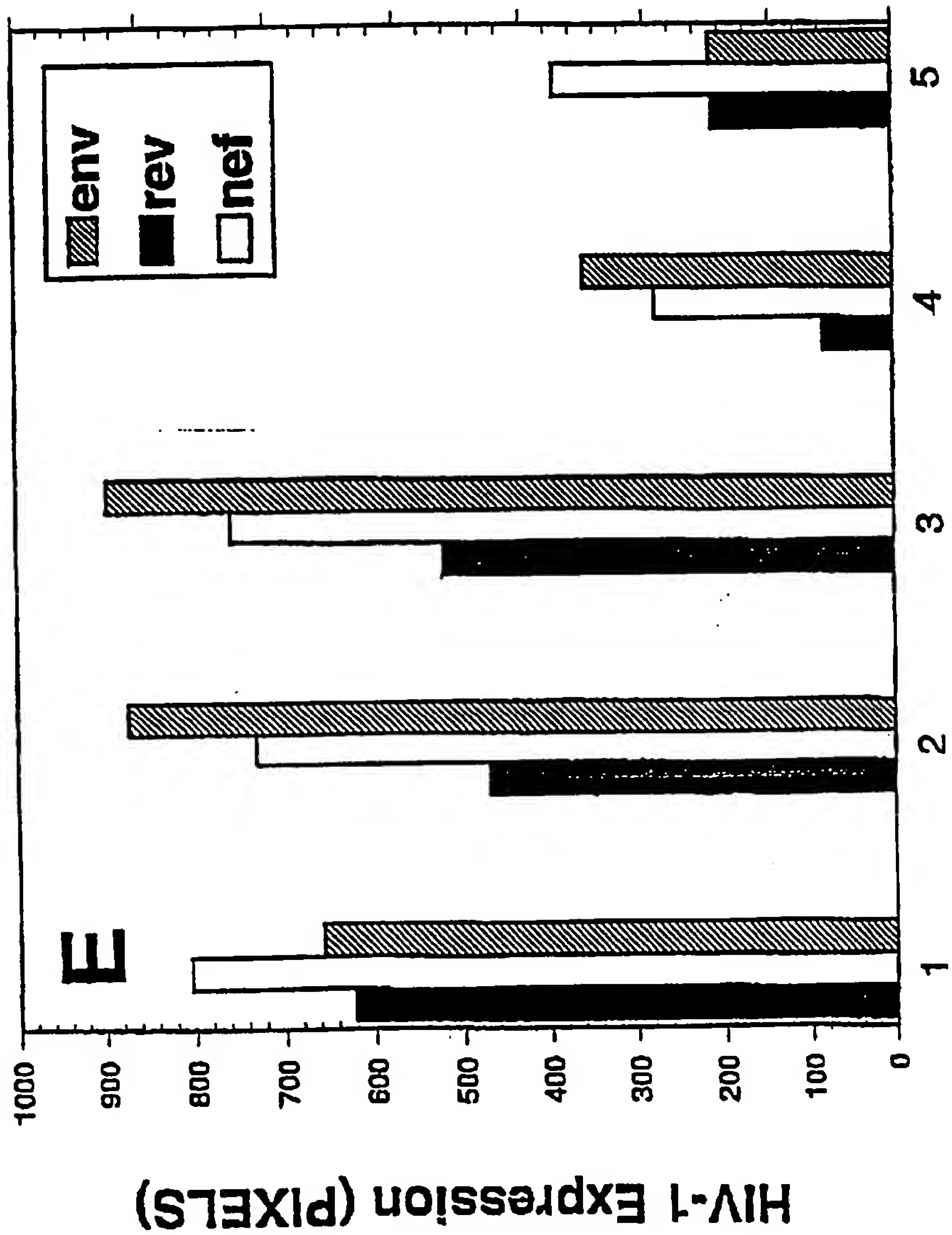
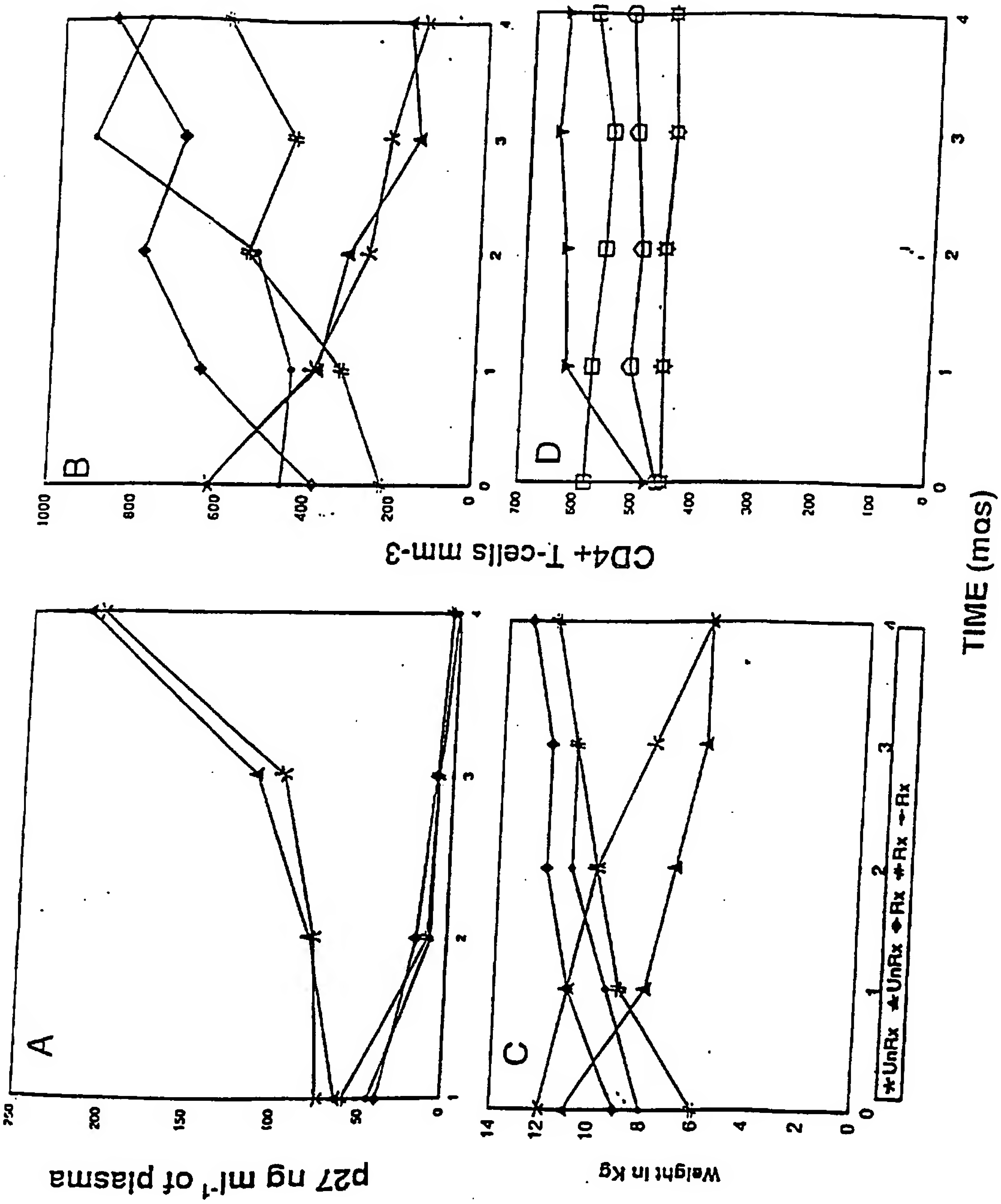
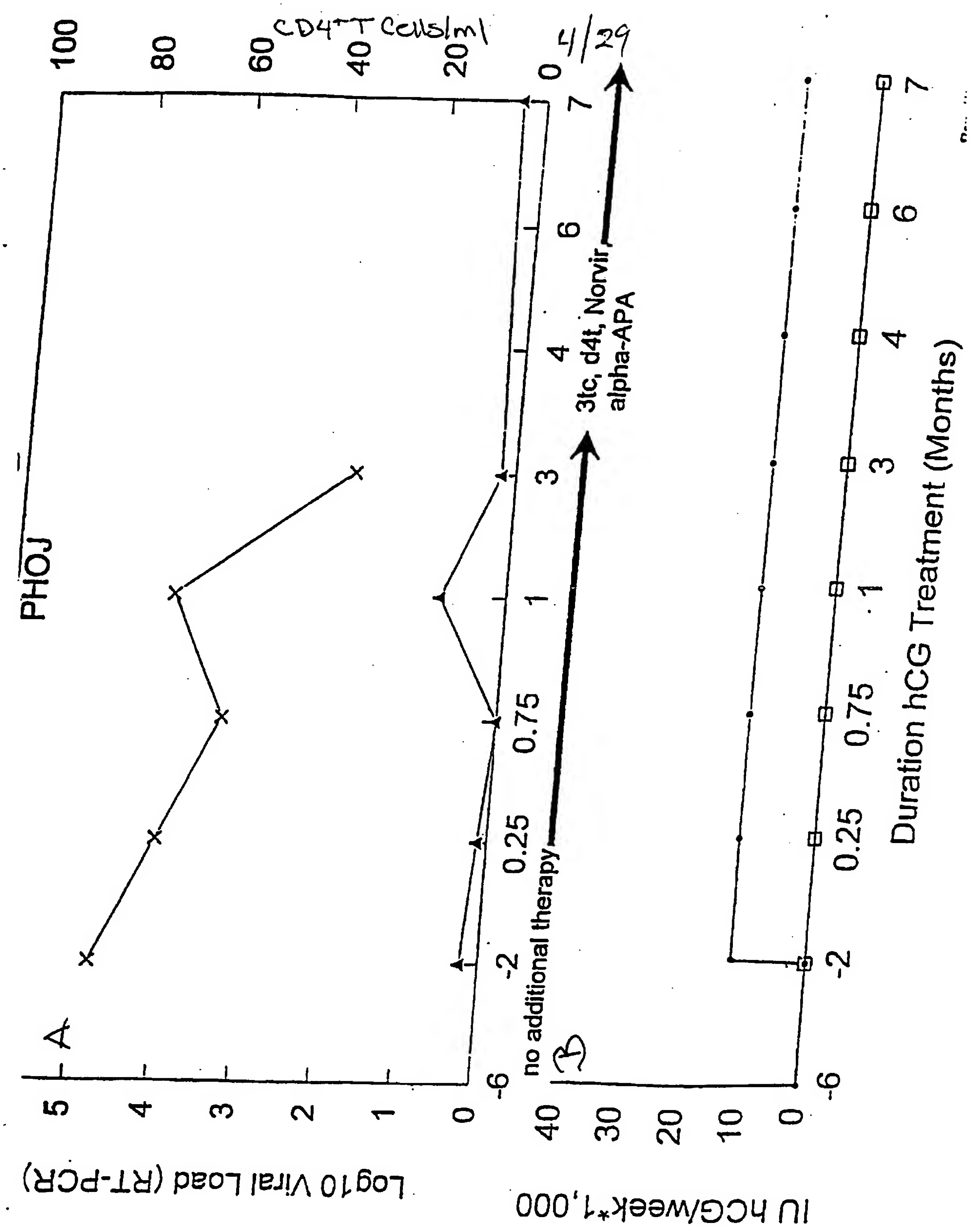
Figure 1E




A
B
C
D
p27 ng ml-1 of plasma
CD4+ T-cells mm-3
Weight in Kg
TIME (mos)
UnRx UnRx Rx Rx Rx

FIGURES 2A-D


PHOJ
A
B
CD4+T Cells/ml
4/29
Log10 Viral Load (RT-PCR)
IU hCG/week*1,000
no additional therapy
3tc, d4t, Norvir
alpha-APA
Duration hCG Treatment (Months)

Figures 3A&B

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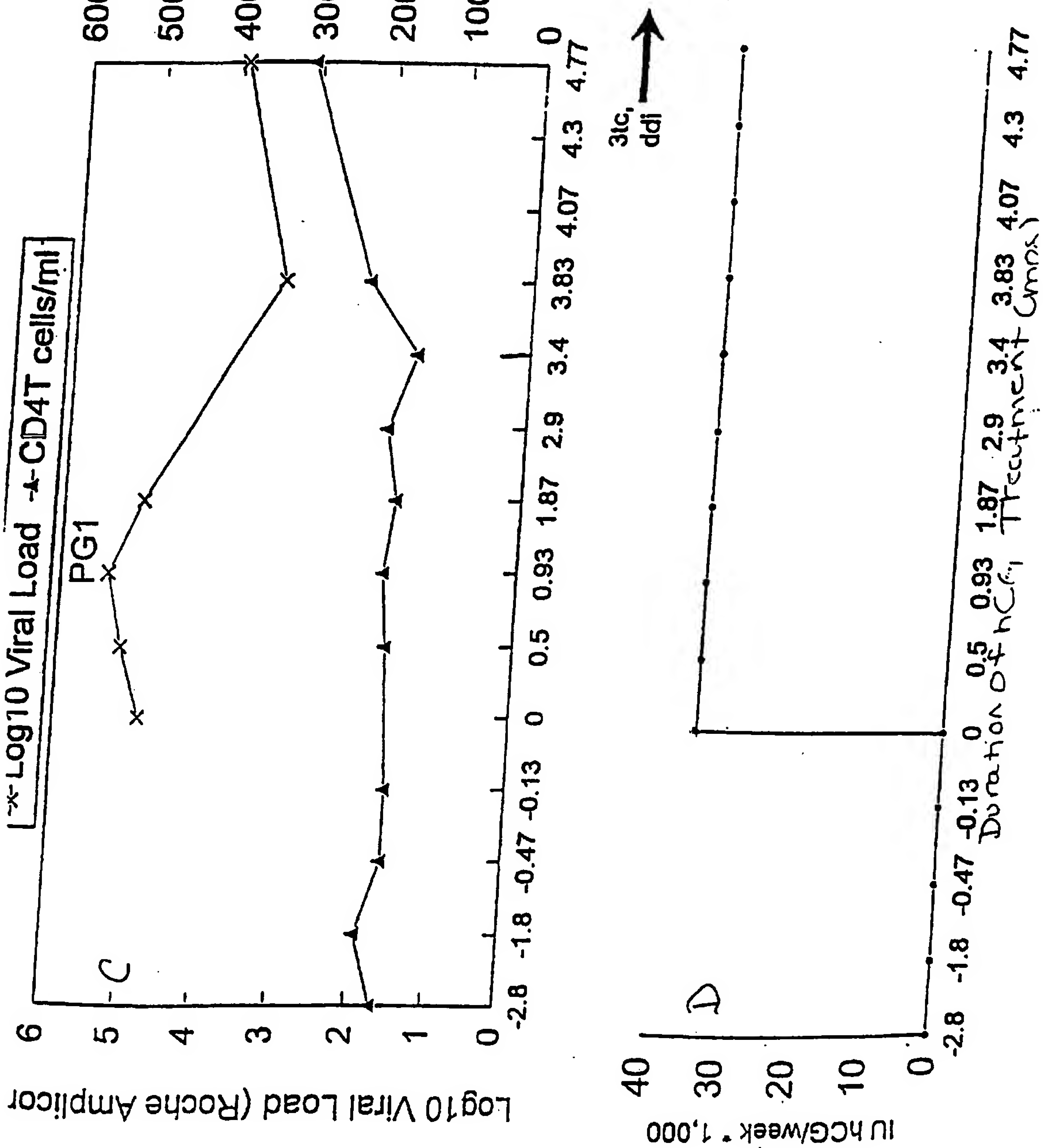
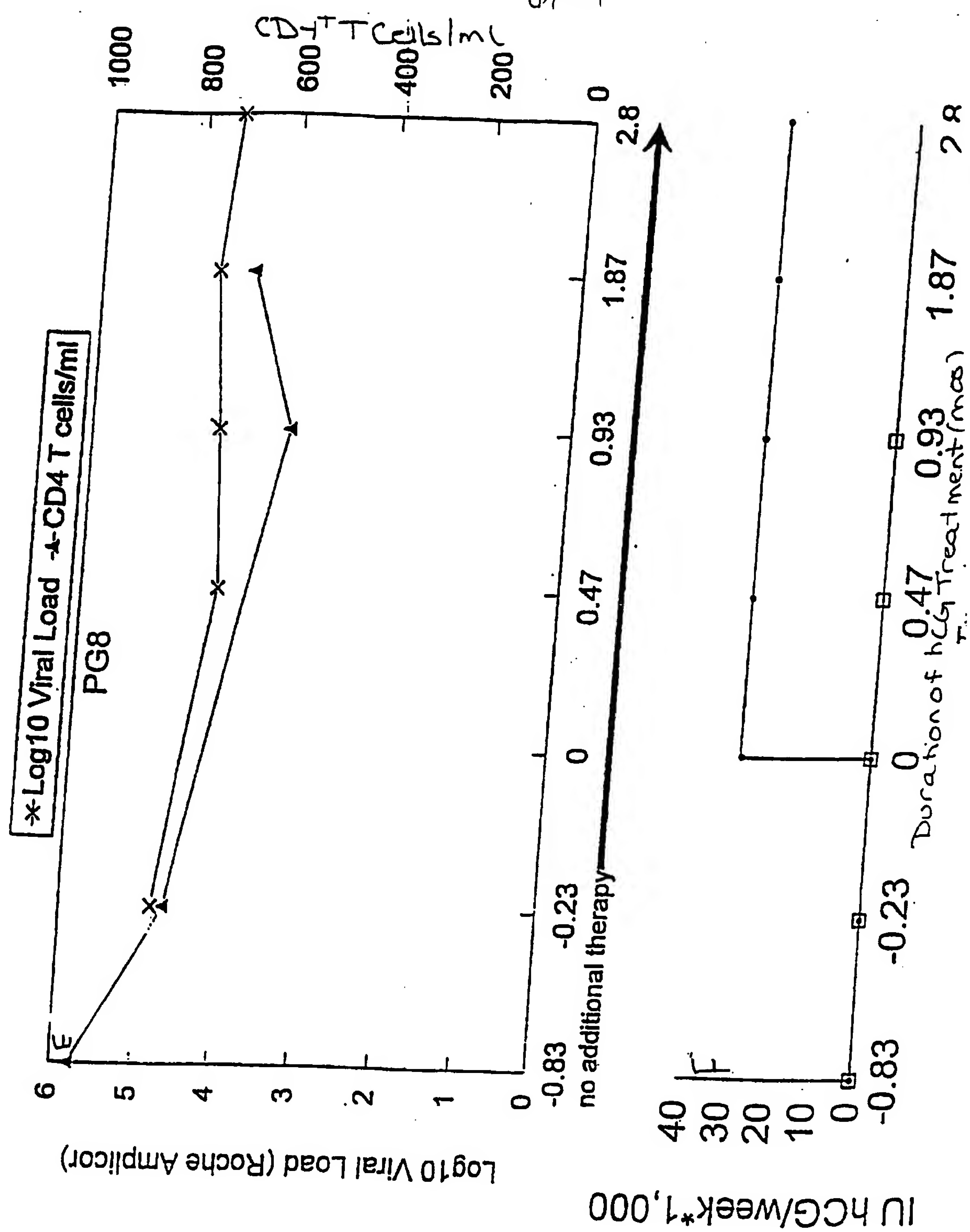
PCT/US 97/11202

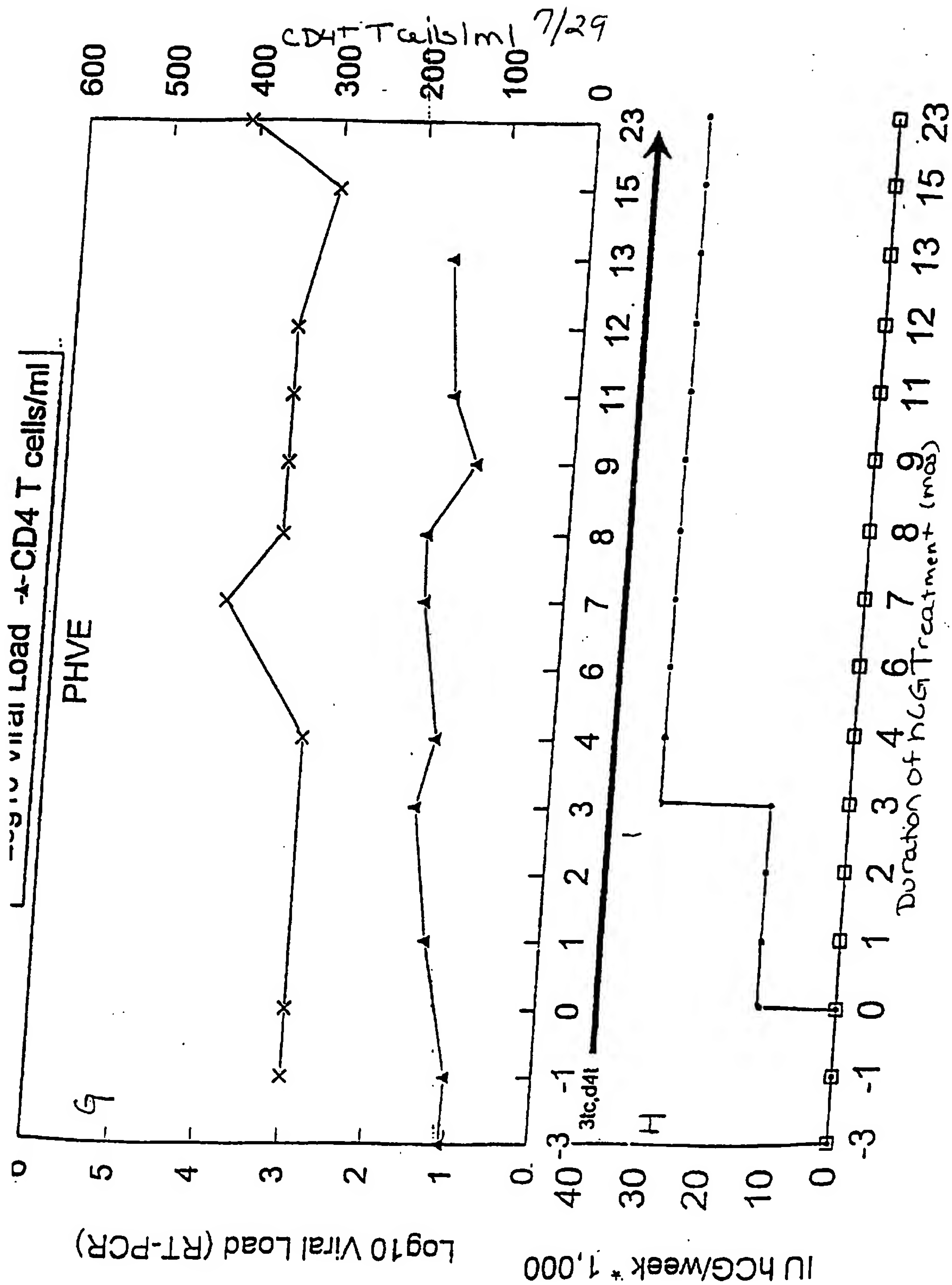


Figures 3C and D



E
PG8
Log10 Viral Load
CD4 T cells/ml
CD4+ T Cells/mL
1000
800
600
400
200
0
6
5
4
3
2
1
0
Log10 Viral Load (Roche Amplicor)
-0.83
-0.23
0
0.47
0.93
1.87
2.8
no additional therapy
F
40
30
20
10
0
IU hCG/week*1,000
Duration of hCG Treatment (mos)

Figures 3E&F



Figures 3G and H

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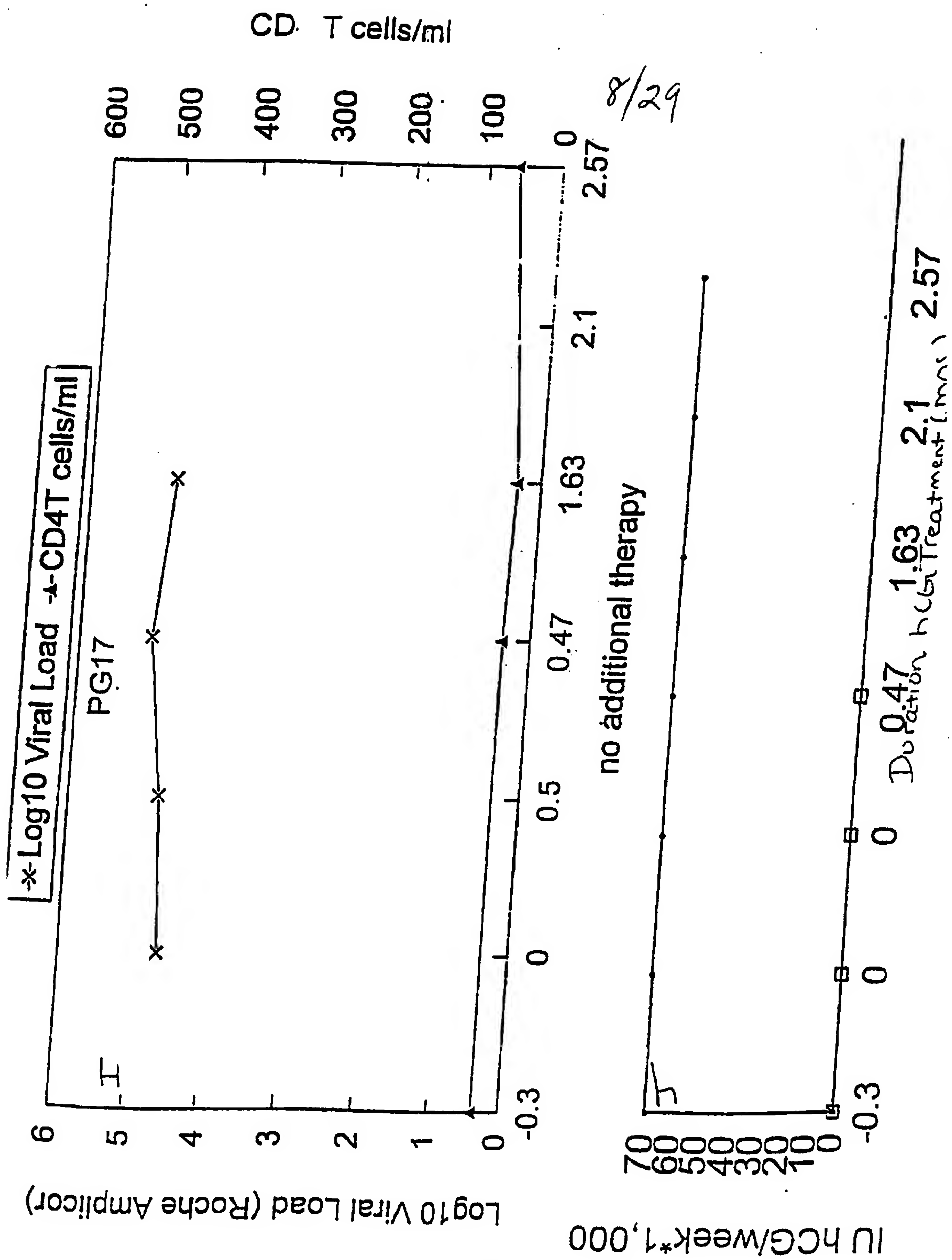
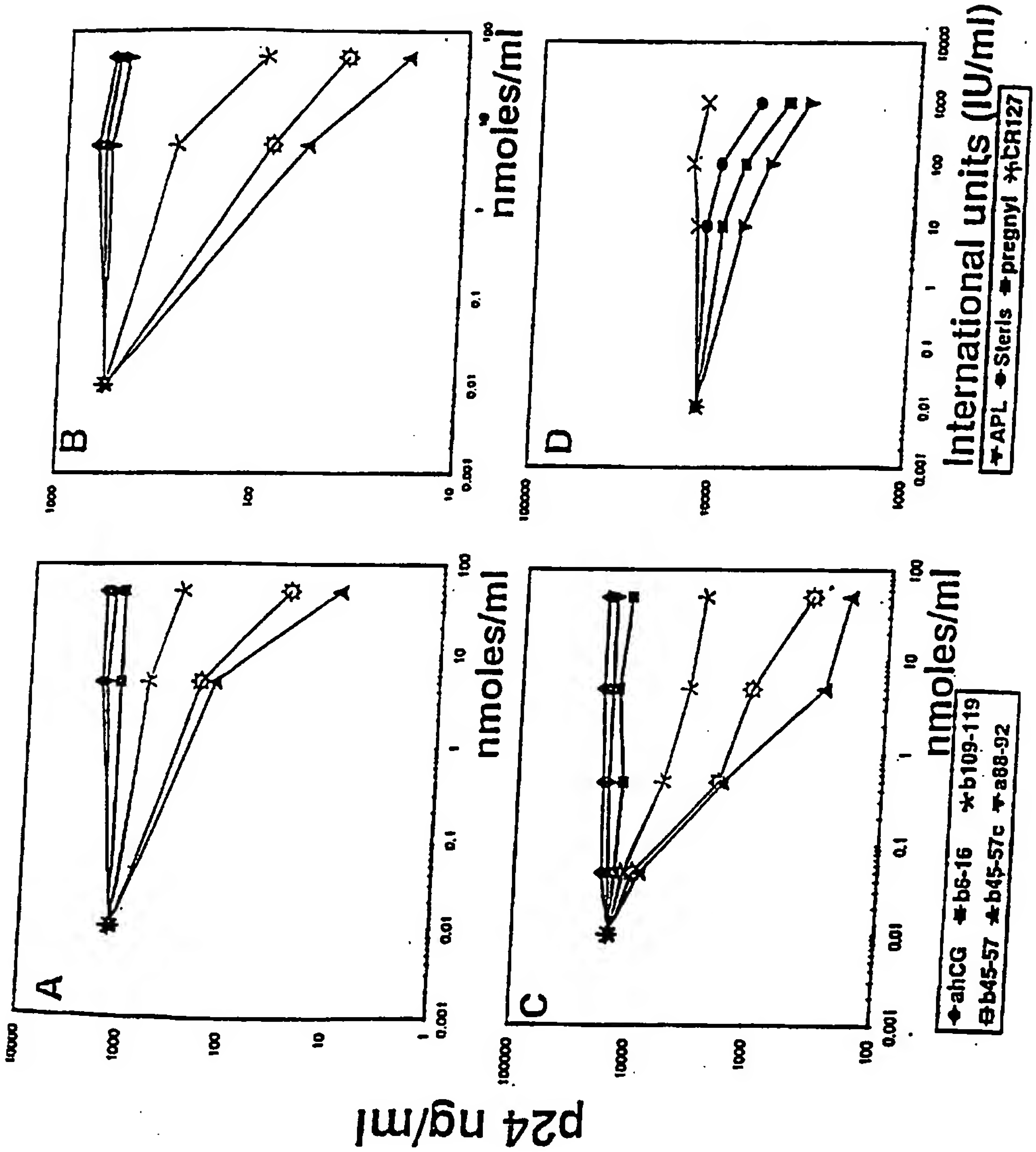


Figures 3I and J


A
B
C
D
p24 ng/ml
nmoles/ml
nmoles/ml
nmoles/ml
International units (IU/ml)
ahCG
b6-16
b109-119
b45-57
b45-57c
a88-92
APL
Steris
pregnyl
CR127

FIGURES 4A-D

FIGURES 5A-C

Effect of preparation of hCG on HIV-LTR activity

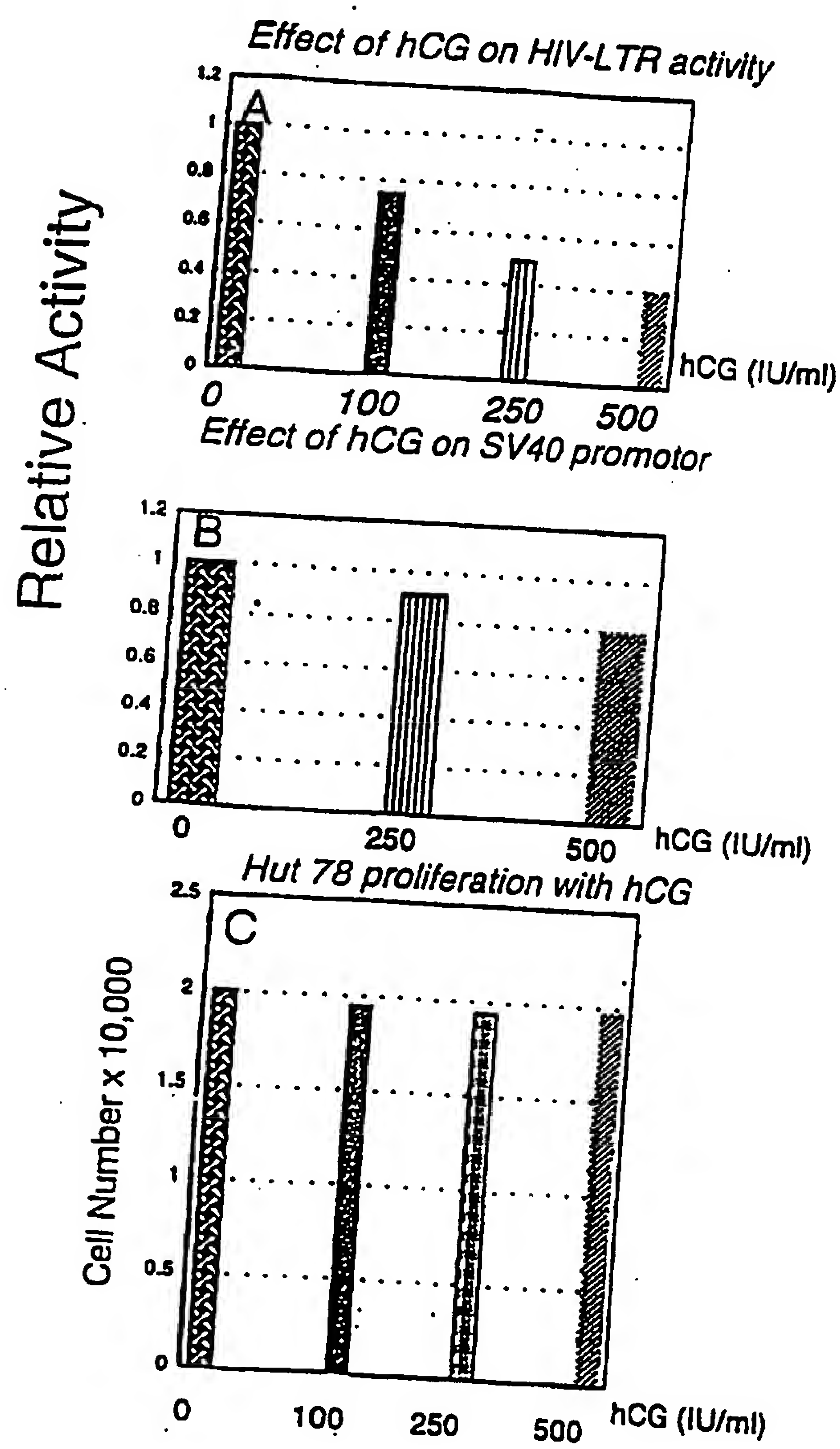


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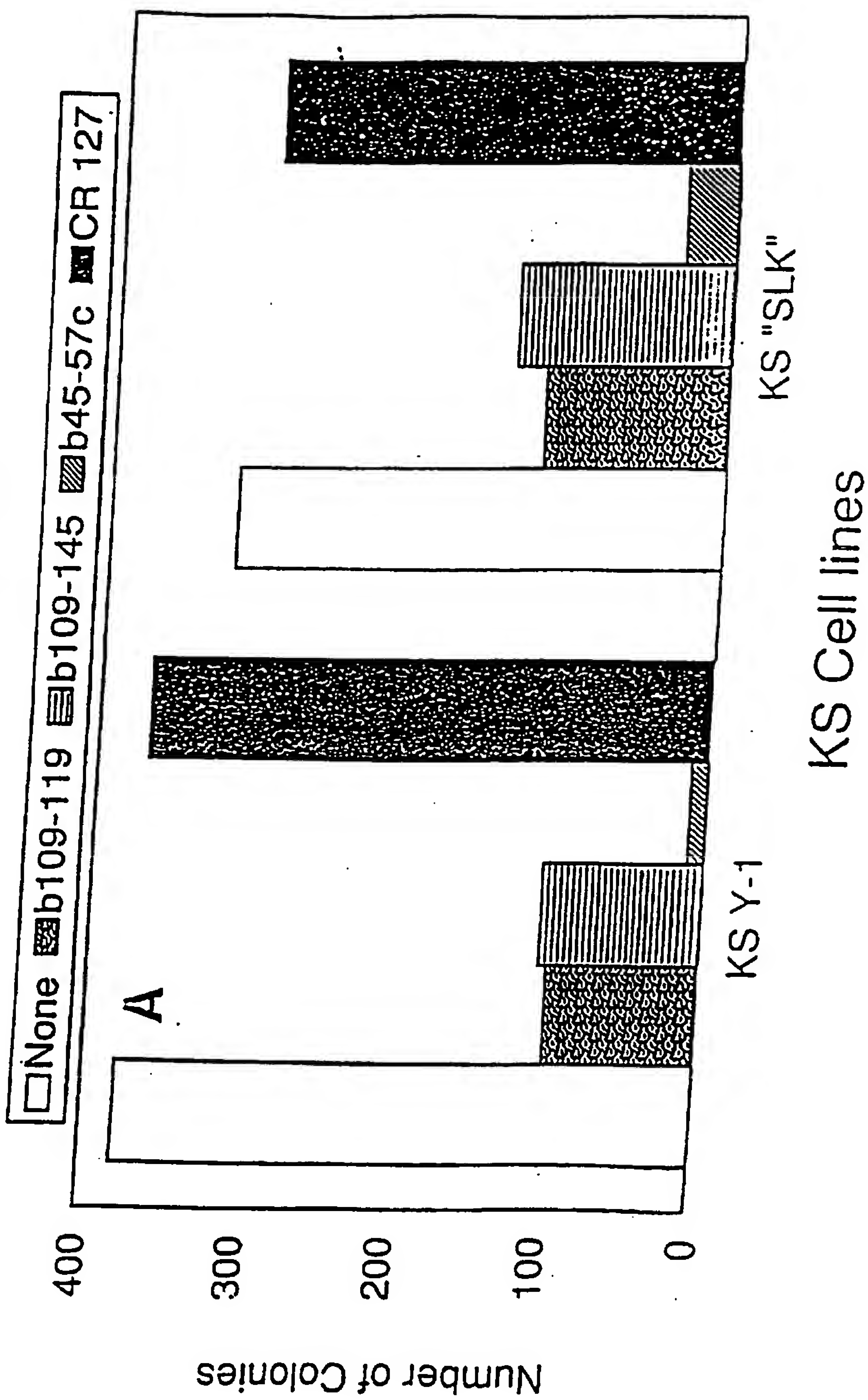
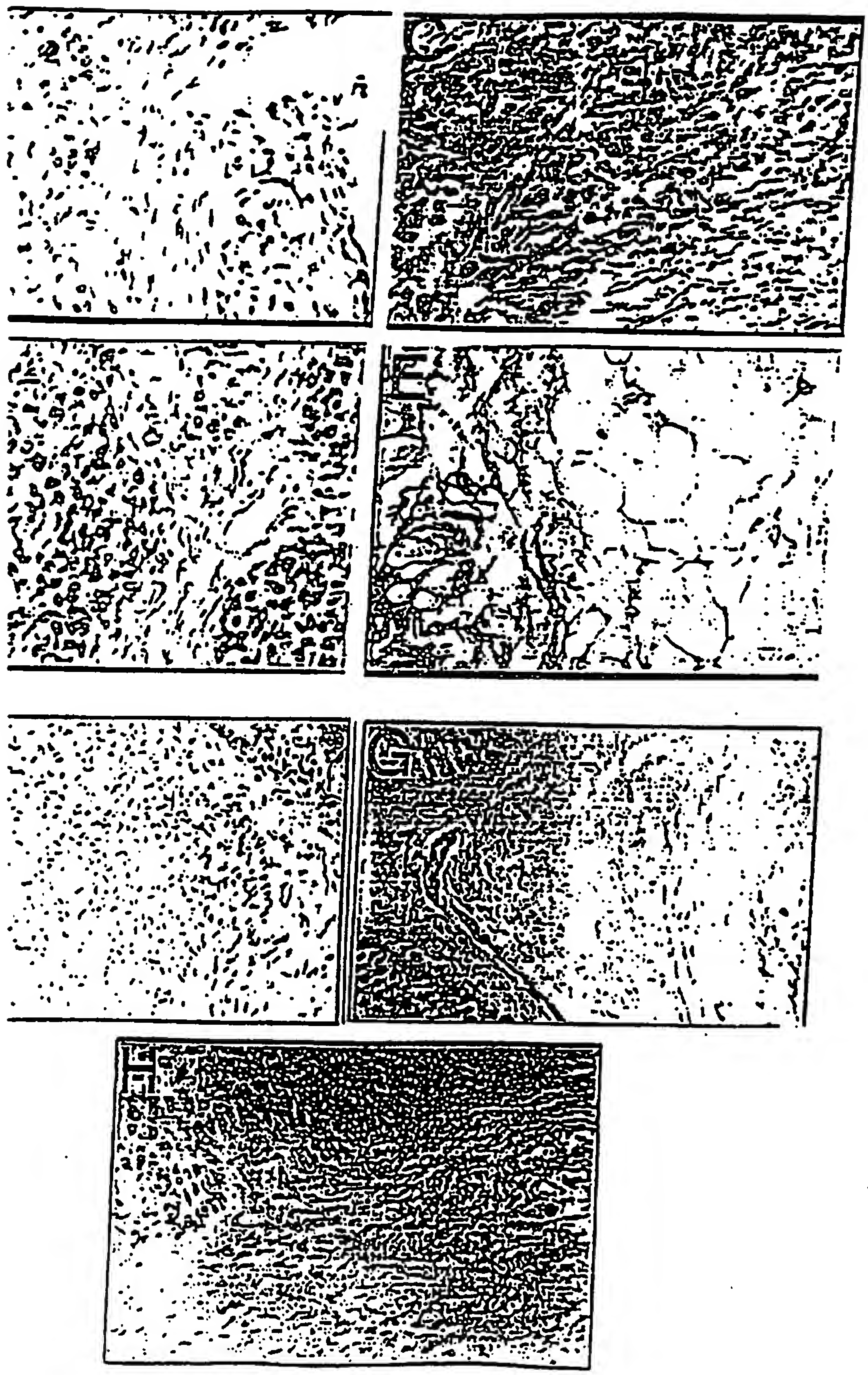
FIGURE 6A



FIGURES 6B-H



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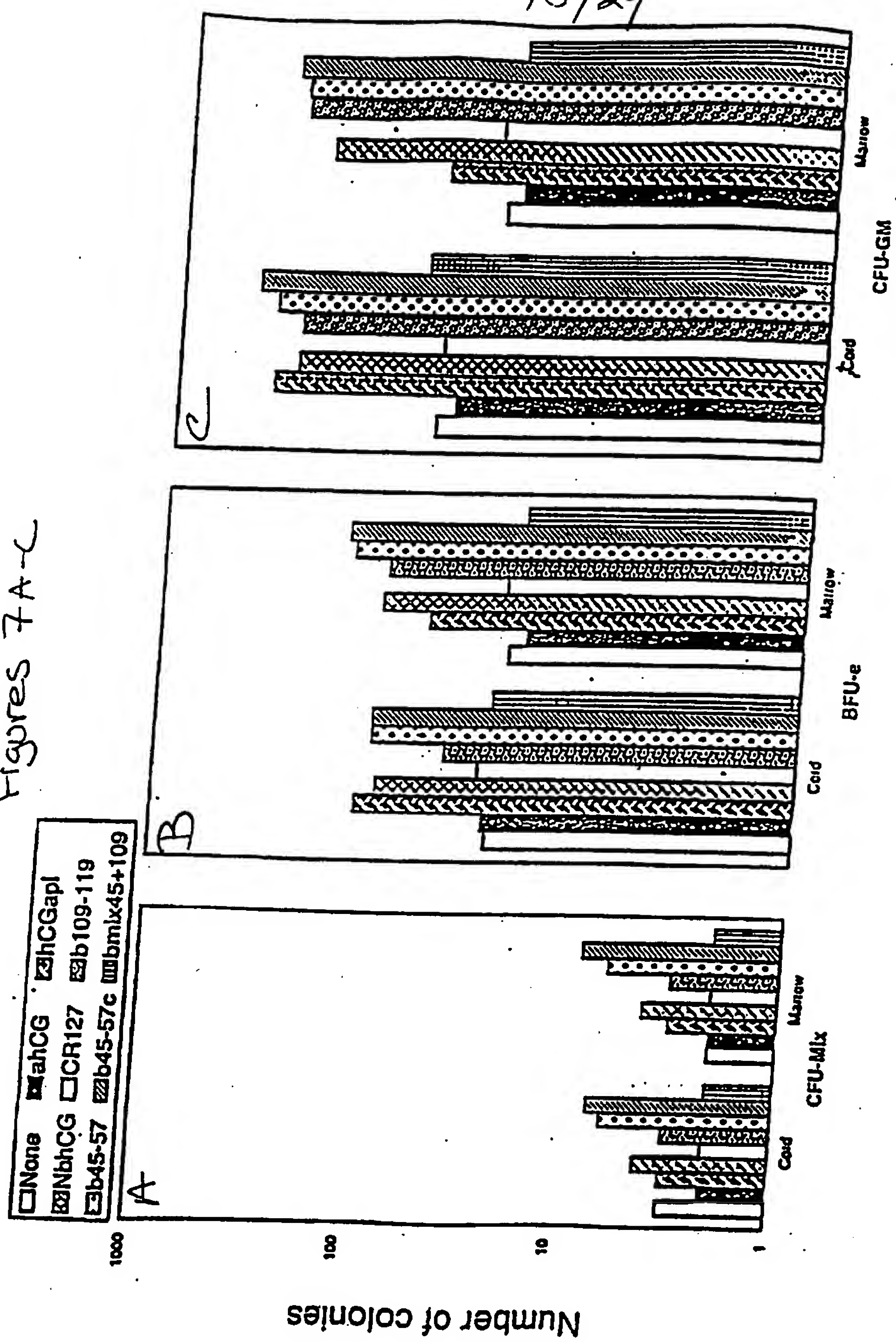
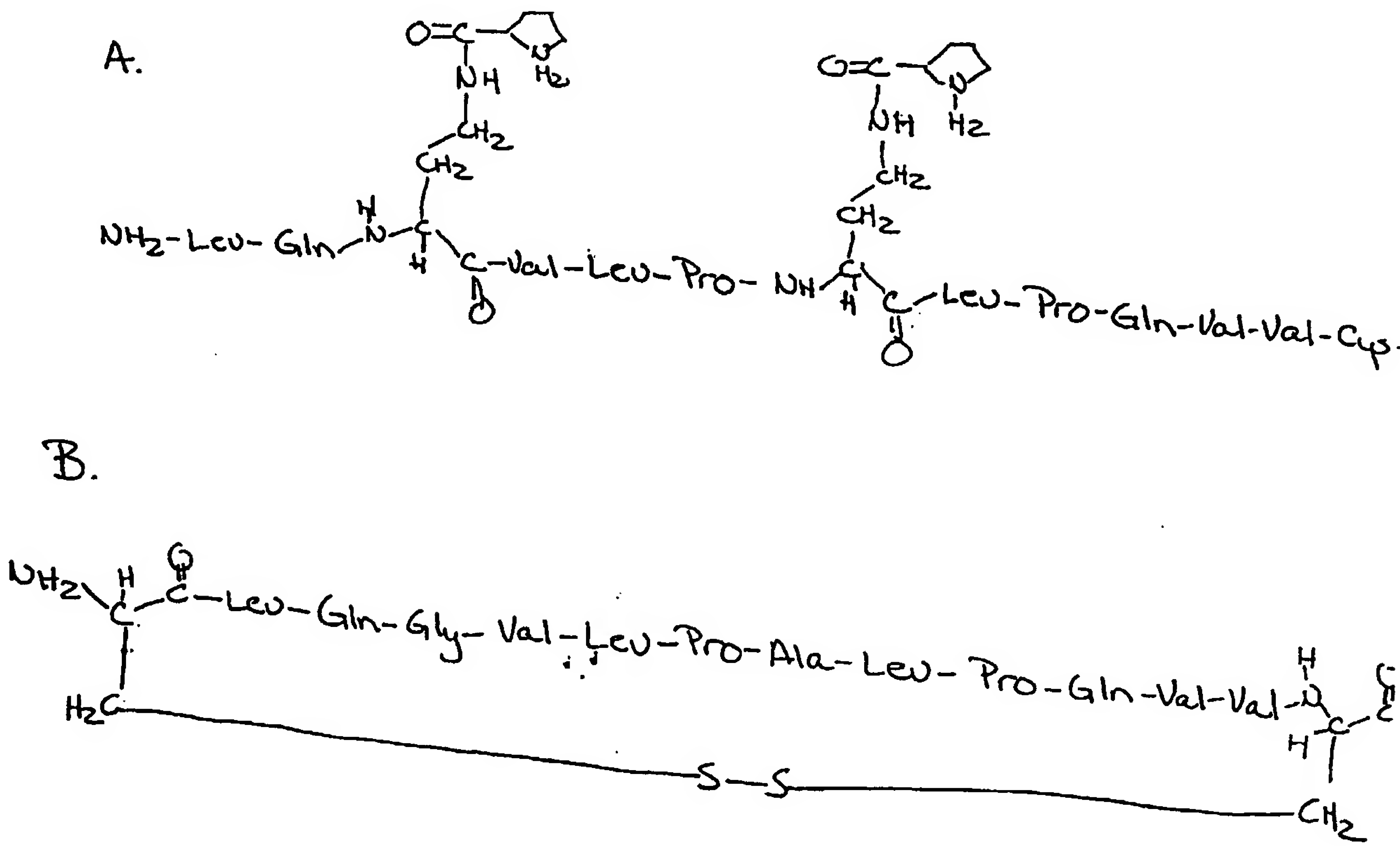


Figures 7A-C

FIGURE 8

```
AGACAAGGCA GGGGACGCAC CAAGG ATG GAG ATG TTC CAG GGG CTG CTG CTG      52
                            Met Glu Met Phe Gln Gly Leu Leu Leu
                            -20                 -15
TTG CTG CTG CTG AGC ATG GGC GGG ACA TGG GCA TCC AAG GAG CCG CTT     100
Leu Leu Leu Leu Ser Met Gly Gly Thr Trp Ala Ser Lys Glu Pro Leu
    -10                 -5                  1               5
CGG CCA CGG TGC CGC CCC ATC AAT GCC ACC CTG GCT GTG GAG AAG GAG     148
Arg Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu Ala Val Glu Lys Glu
            10                  15                  20
GGC TGC CCC GTG TGC ATC ACC GTC AAC ACC ACC ATC TGT GCC GGC TAC     196
Gly Cys Pro Val Cys Ile Thr Val Asn Thr Thr Ile Cys Ala Gly Tyr
        25                  30                  35
TGC CCC ACC ATG ACC CGC GTG CTG CAG GGG GTC CTG CCG GCC CTG CCT     244
Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro
    40                  45                  50
CAG GTG GTG TGC AAC TAC CGC GAT GTG CGC TTC GAG TCC ATC CGG CTC     292
Gln Val Val Cys Asn Tyr Arg Asp Val Arg Phe Glu Ser Ile Arg Leu
    55                  60                  65
CCT GGC TGC CCG CGC GGC GTG AAC CCC GTG GTC TCC TAC GCC GTG GCT     340
Pro Gly Cys Pro Arg Gly Val Asn Pro Val Val Ser Tyr Ala Val Ala
70                  75                  80                  85
CTC AGC TGT CAA TGT GCA CTC TGC CGC CGC AGC ACC ACT GAC TGC GGG     388
Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg Ser Thr Thr Asp Cys Gly
                90                  95                  100
GGT CCC AAG GAC CAC CCC TTG ACC TGT GAT GAC CCC CGC TTC CAG GAC     436
Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp
            105                 110                 115
TCC TCT TCC TCA AAG GCC CCT CCC CCC AGC CTT CCA AGC CCA TCC CGA     484
Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg
        120                 125                 130
CTC CCG GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA TAAAGGCTTC          530
Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln
    135                 140                 145
TCAATCCGC                                                           539
```

FIGURES 9A&B



Figures 10A-F

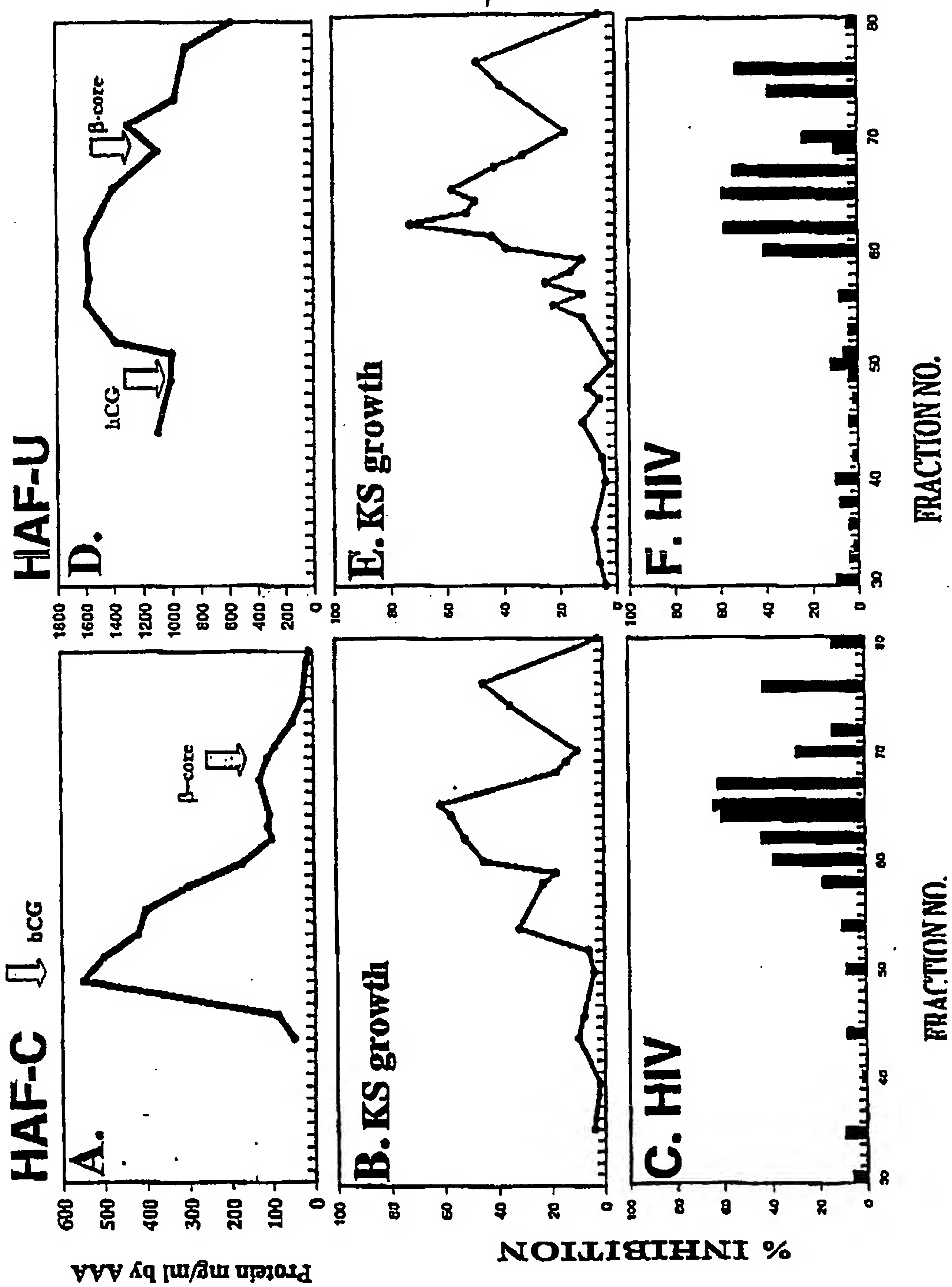
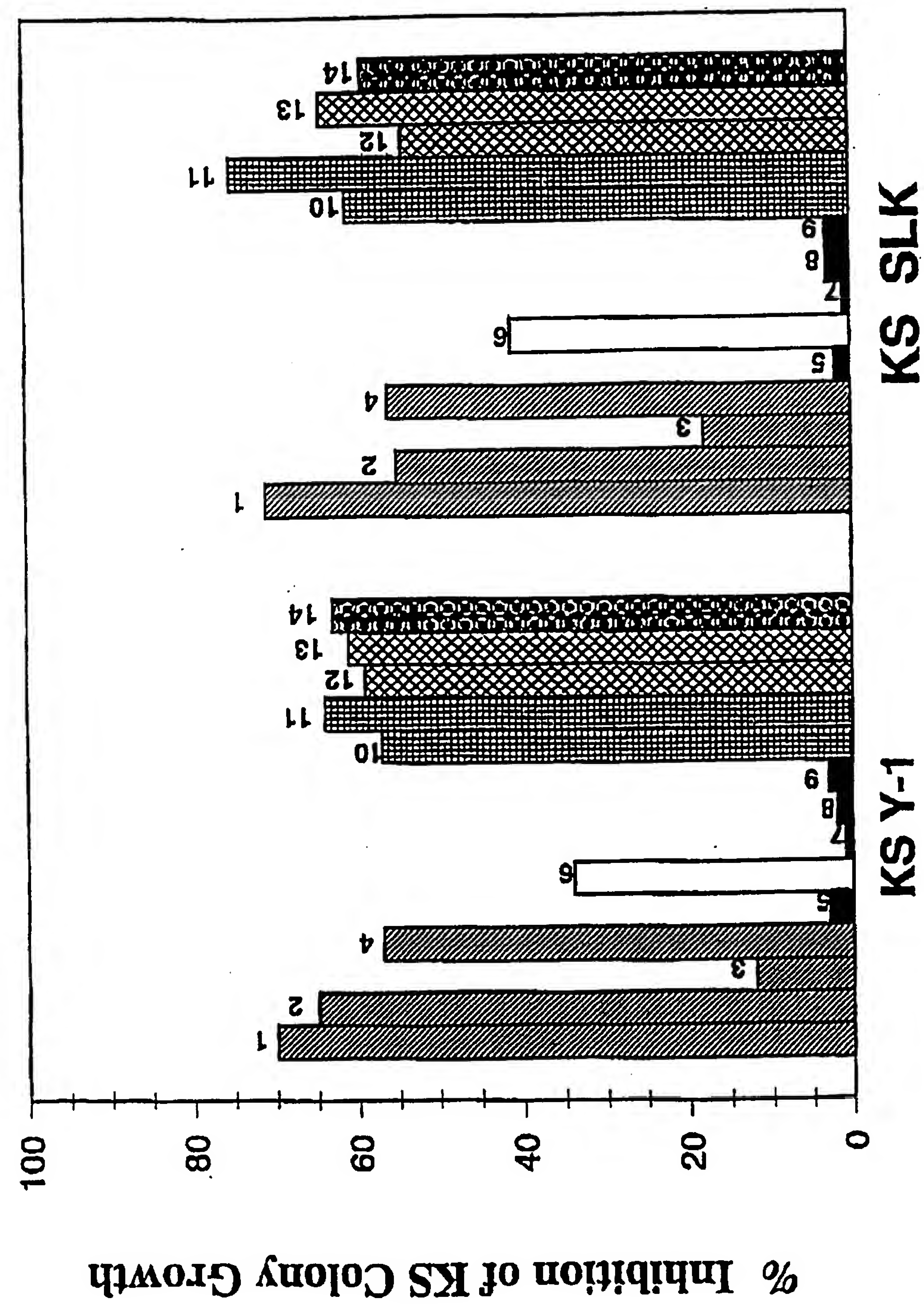


Figure 11


% Inhibition of KS Colony Growth
0
20
40
60
80
100
KS Y-1
KS SLK
1
2
3
4
5
6
7
8
9
10
11
12
13
14

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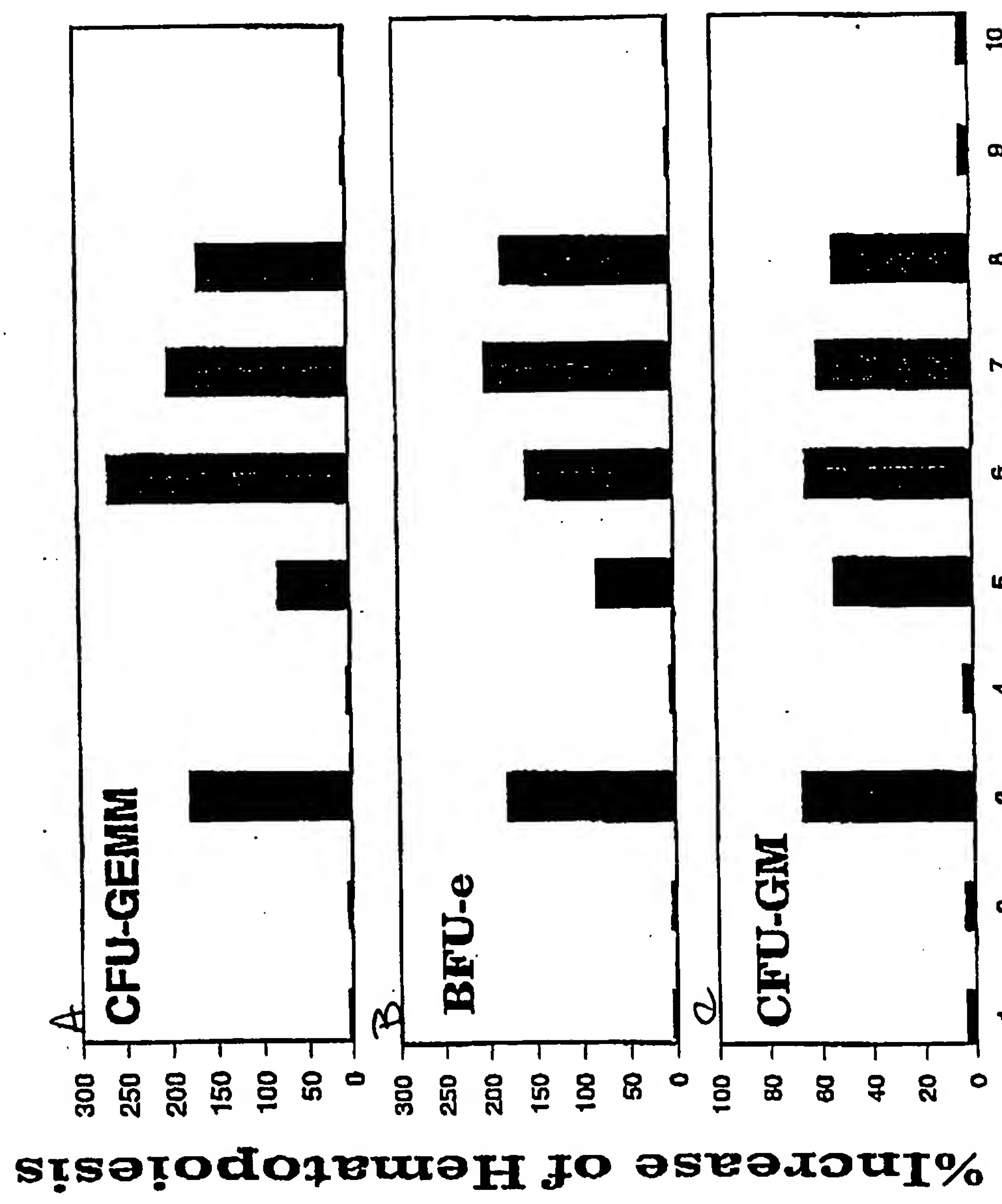
Figures 12 A - C



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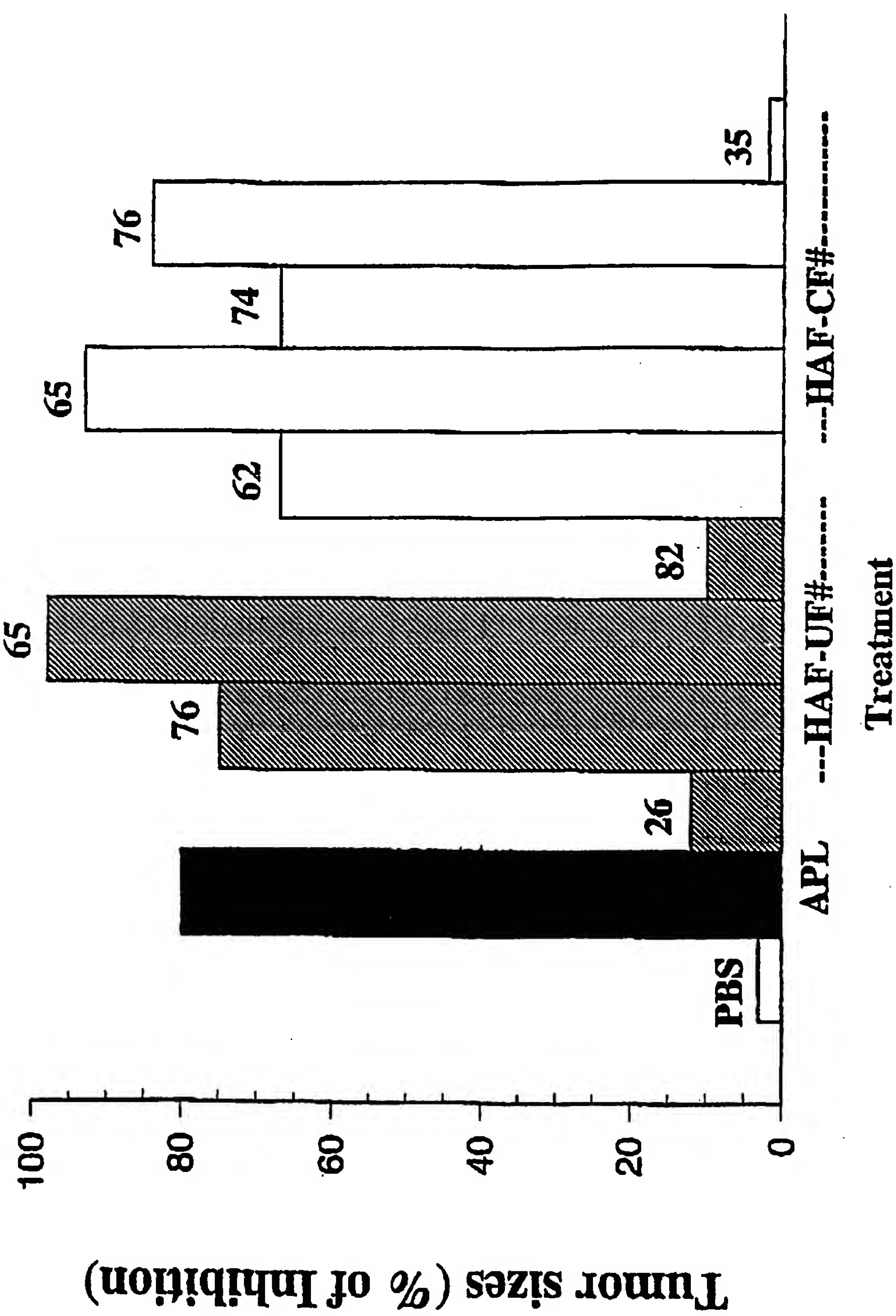

Figure 13

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Figure 14

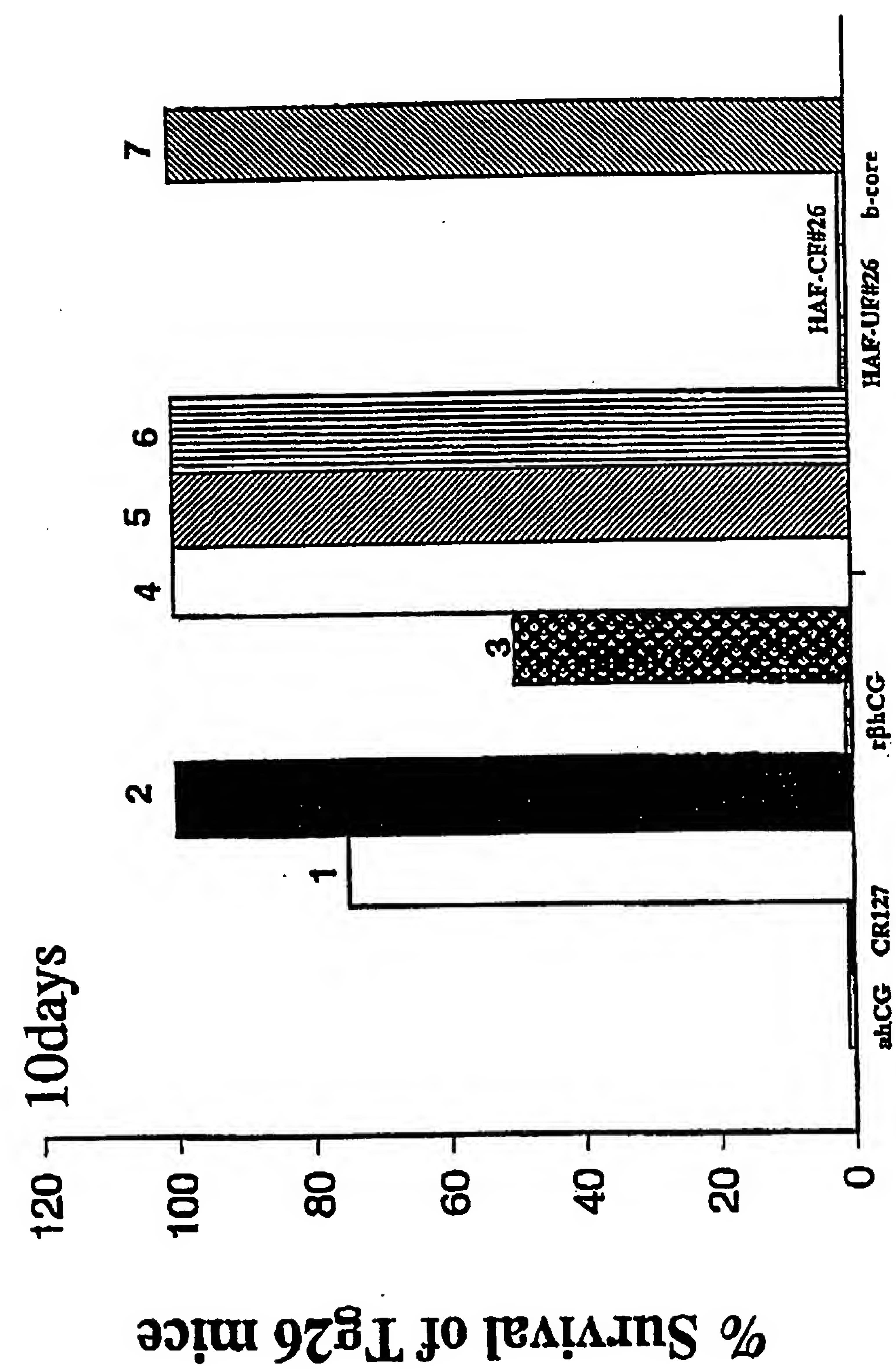


Figure 15A

Viral Load Change after HAF

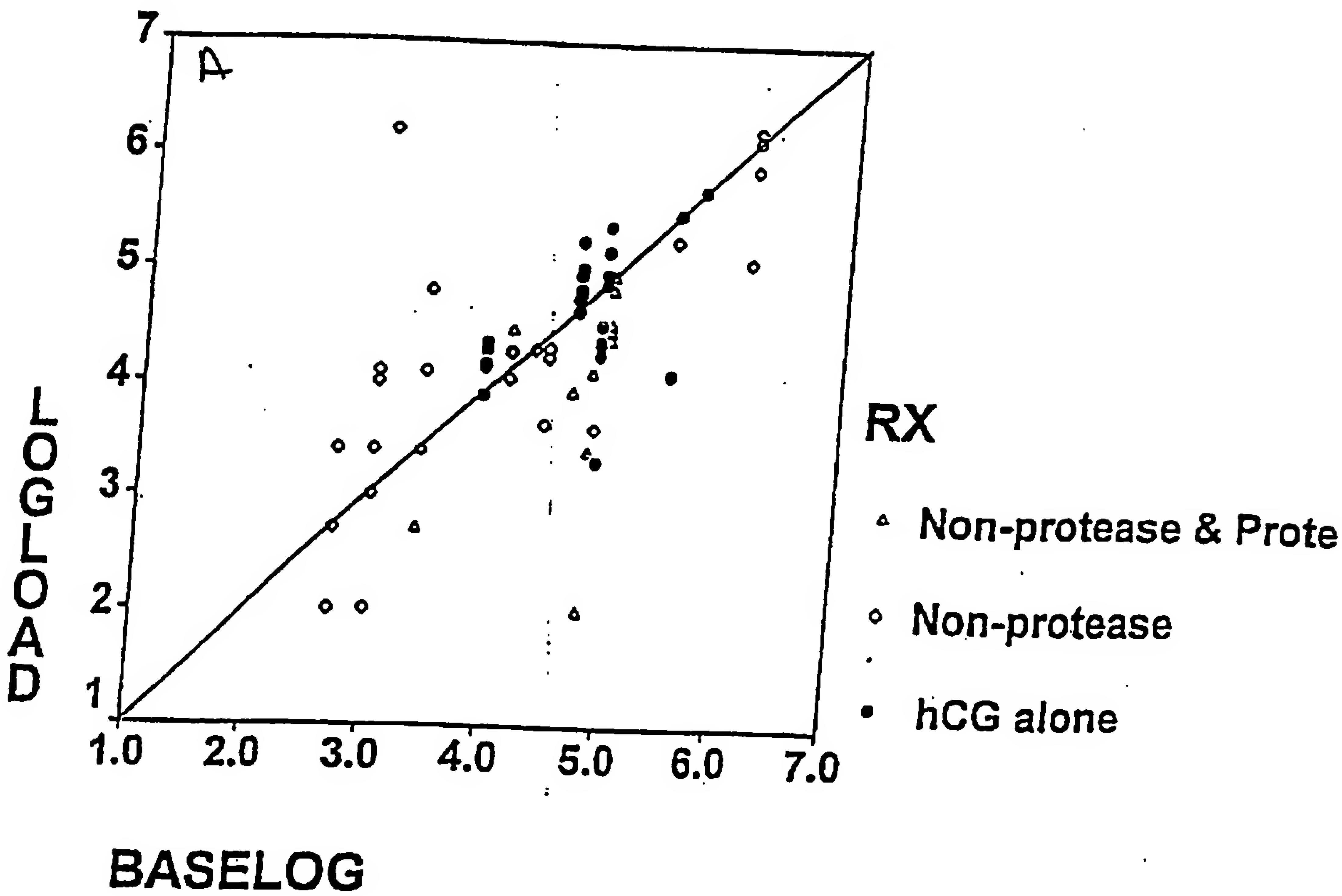


Figure 15B

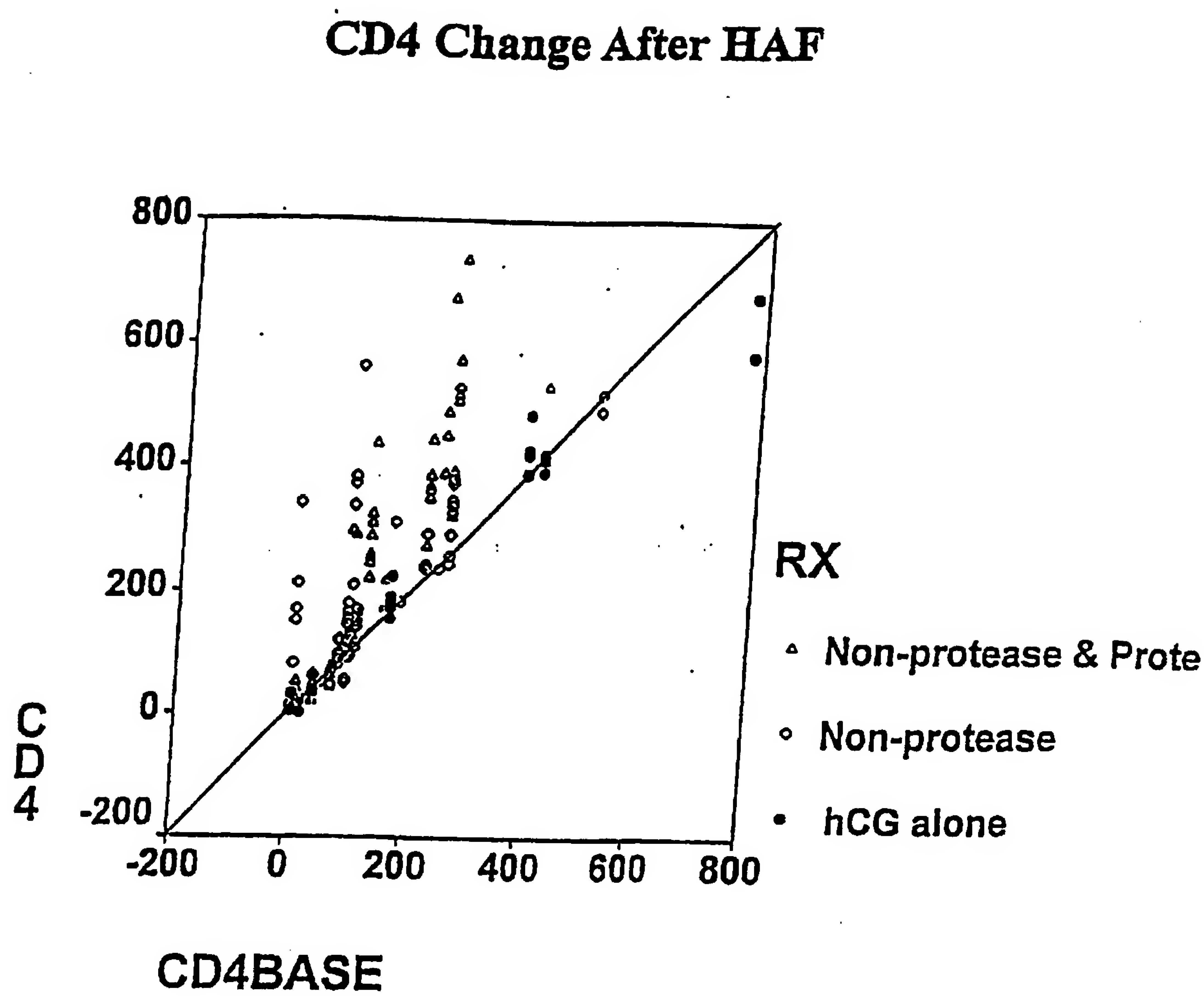
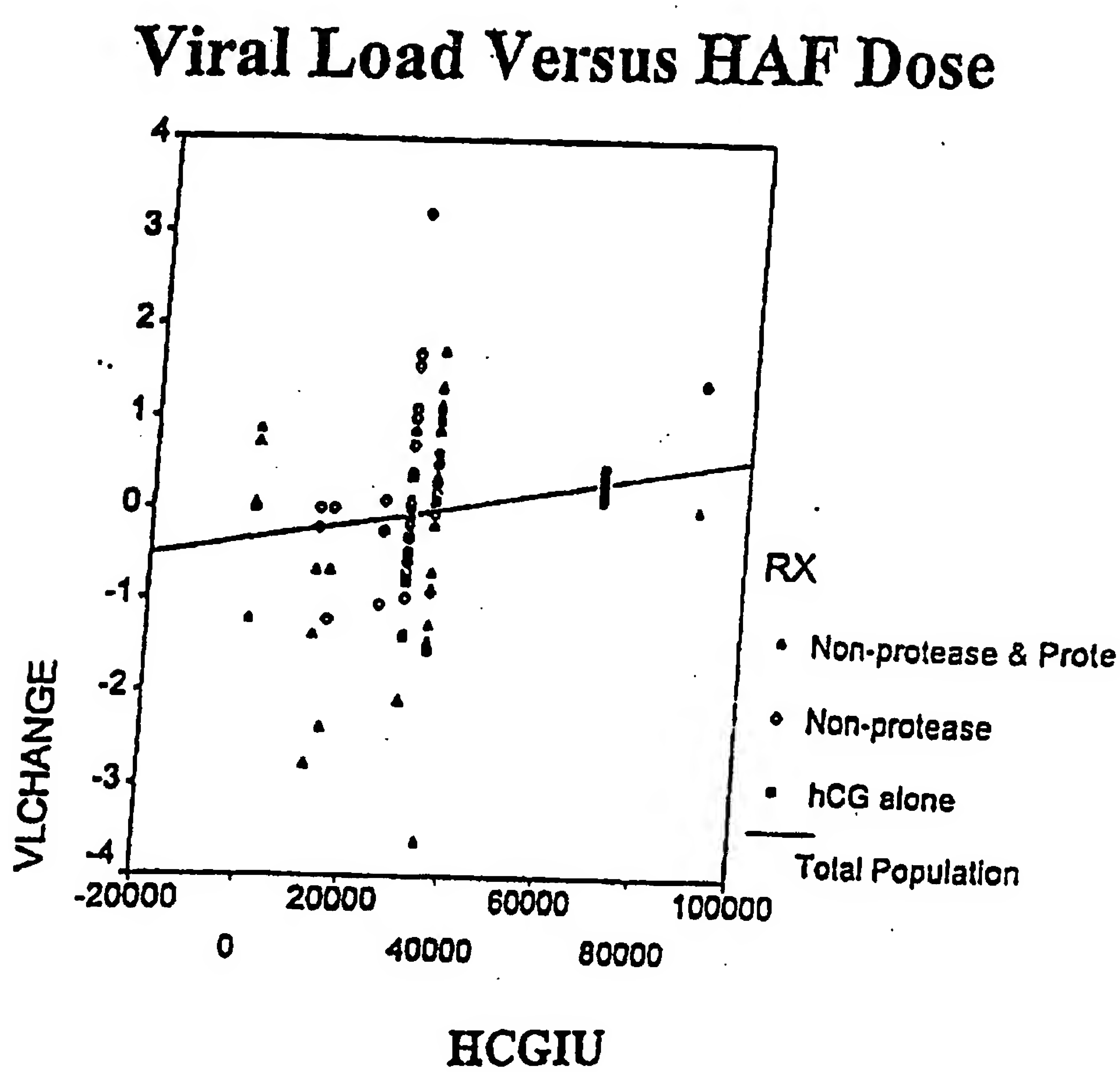
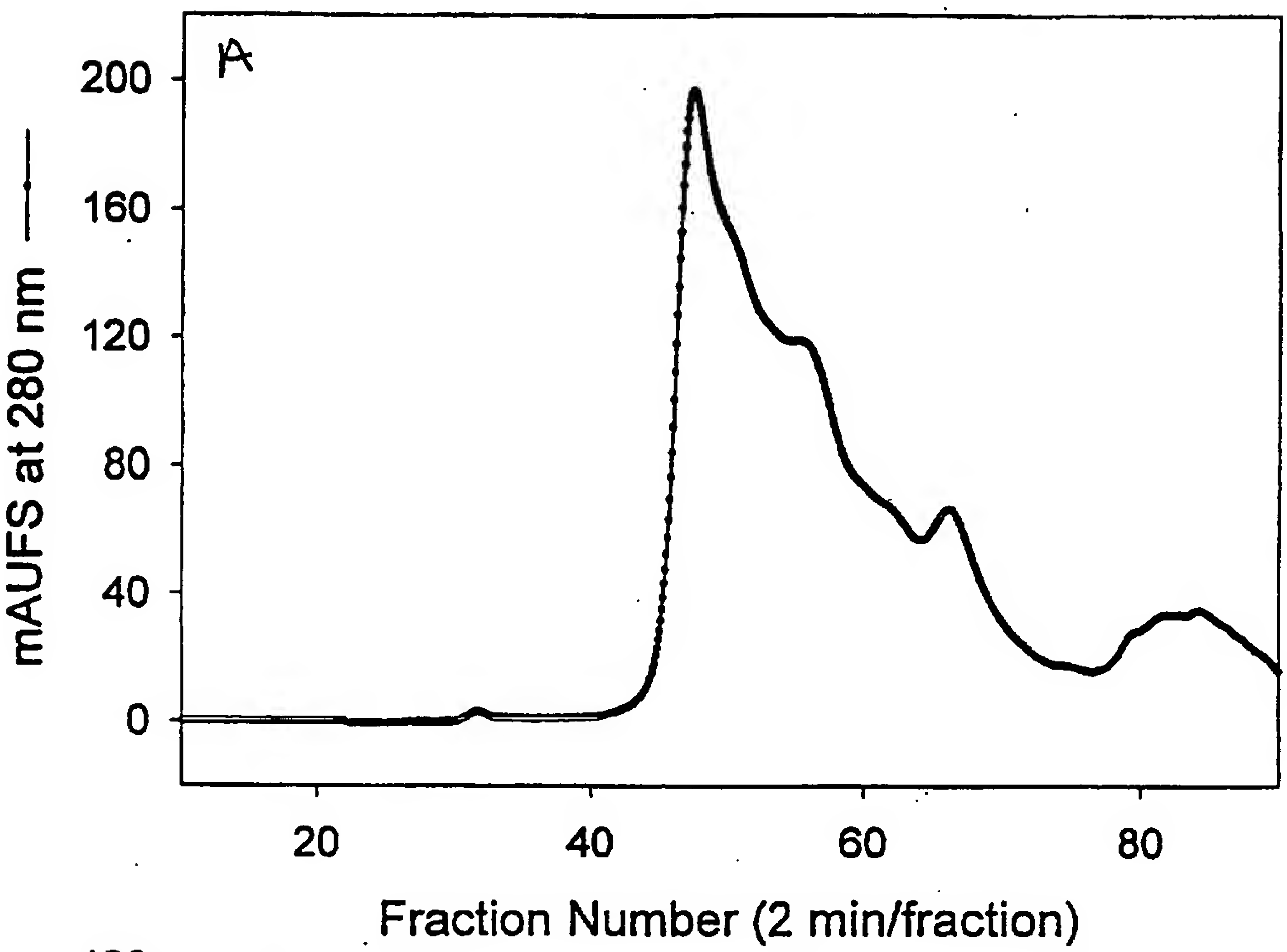

CD4 Change After HAF
800
600
400
200
0
-200
C
D
4
-200
0
200
400
600
800
CD4BASE
RX
Non-protease & Prote
Non-protease
hCG alone

Figure 15C


Viral Load Versus HAF Dose
4
3
2
1
0
-1
-2
-3
-4
VLCHANGE
-20000
0
20000
40000
60000
80000
100000
HCGIU
RX
Non-protease & Prote
Non-protease
hCG alone
Total Population

Figures 16 A and B



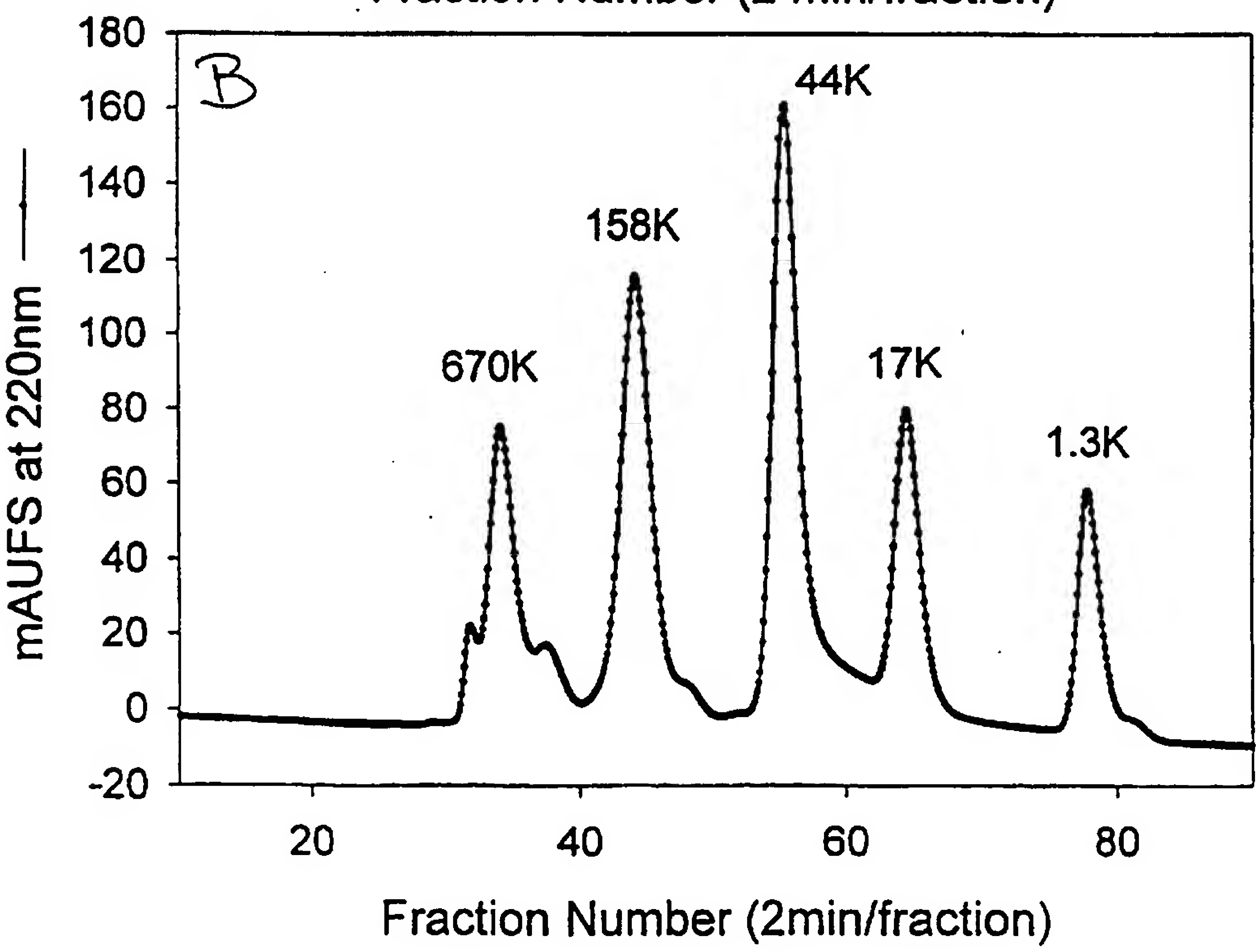
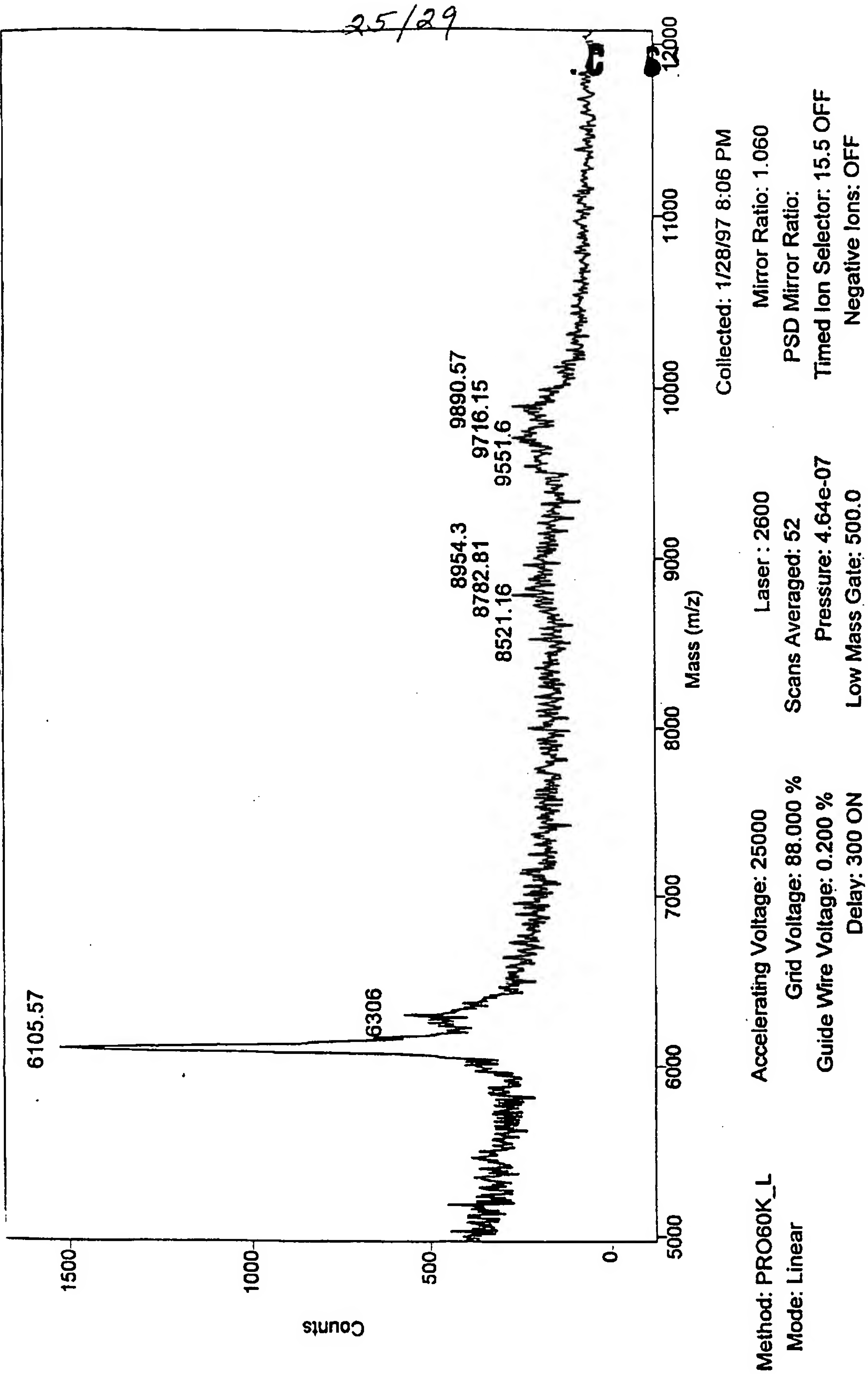


Figure 17A


6105.57
6306
8521.16
8782.81
8954.3
9551.6
9716.15
9890.57
Counts
1500
1000
500
0
5000
6000
7000
8000
9000
10000
11000
12000
Mass (m/z)
Method: PRO60K_L
Mode: Linear
Accelerating Voltage: 25000
Grid Voltage: 88.000 %
Guide Wire Voltage: 0.200 %
Delay: 300 ON
Laser : 2600
Scans Averaged: 52
Pressure: 4.64e-07
Low Mass Gate: 500.0
Collected: 1/28/97 8:06 PM
Mirror Ratio: 1.060
PSD Mirror Ratio:
Timed Ion Selector: 15.5 OFF
Negative Ions: OFF

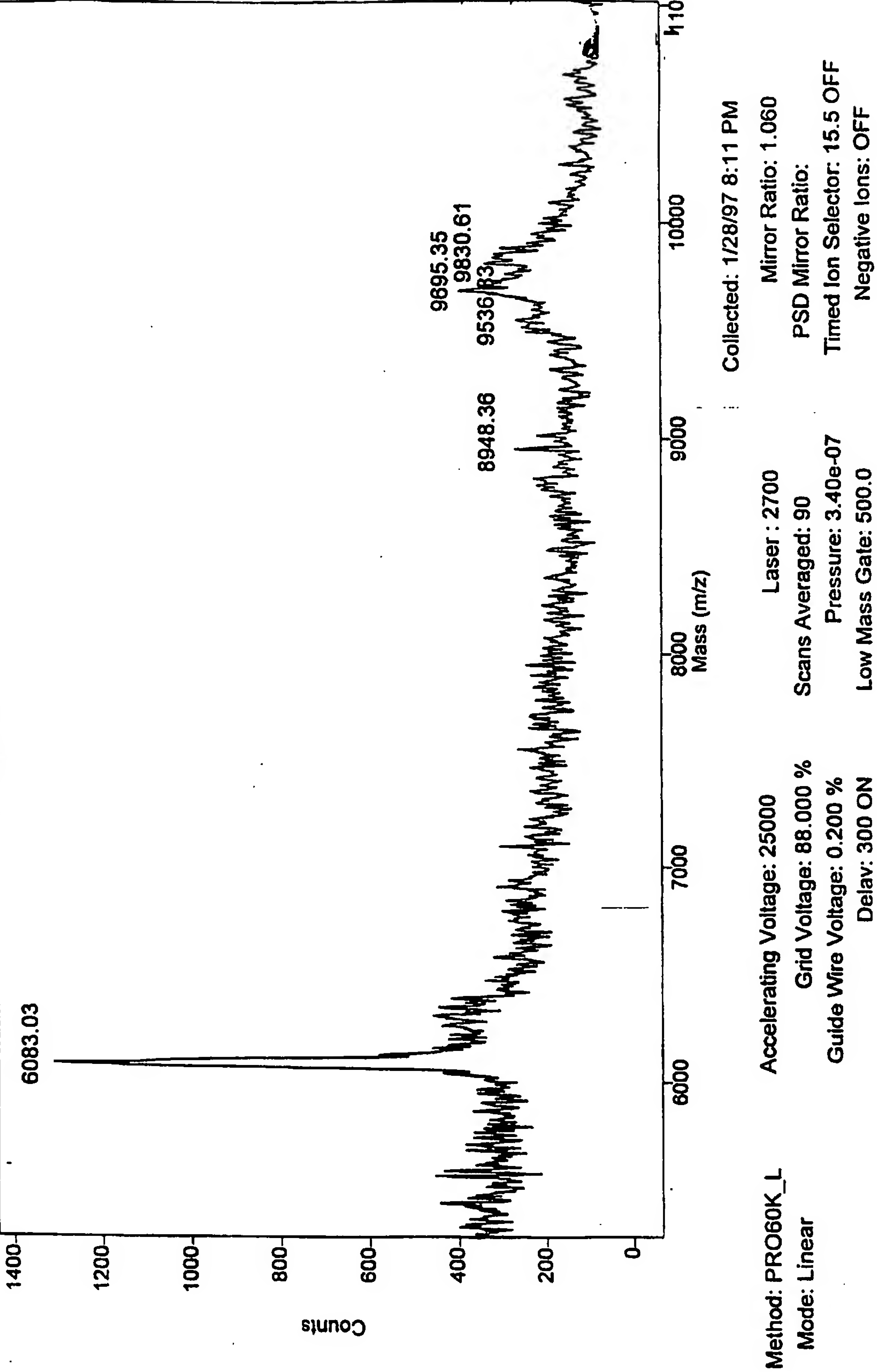

6083.03
8948.36
9536.83
9695.35
9830.61
Counts
1400
1200
1000
800
600
400
200
0
6000
7000
8000
9000
10000
11000
Mass (m/z)
Method: PRO60K_L
Mode: Linear
Accelerating Voltage: 25000
Grid Voltage: 88.000 %
Guide Wire Voltage: 0.200 %
Delay: 300 ON
Laser : 2700
Scans Averaged: 90
Pressure: 3.40e-07
Low Mass Gate: 500.0
Collected: 1/28/97 8:11 PM
Mirror Ratio: 1.060
PSD Mirror Ratio:
Timed Ion Selector: 15.5 OFF
Negative Ions: OFF

Figure 17B

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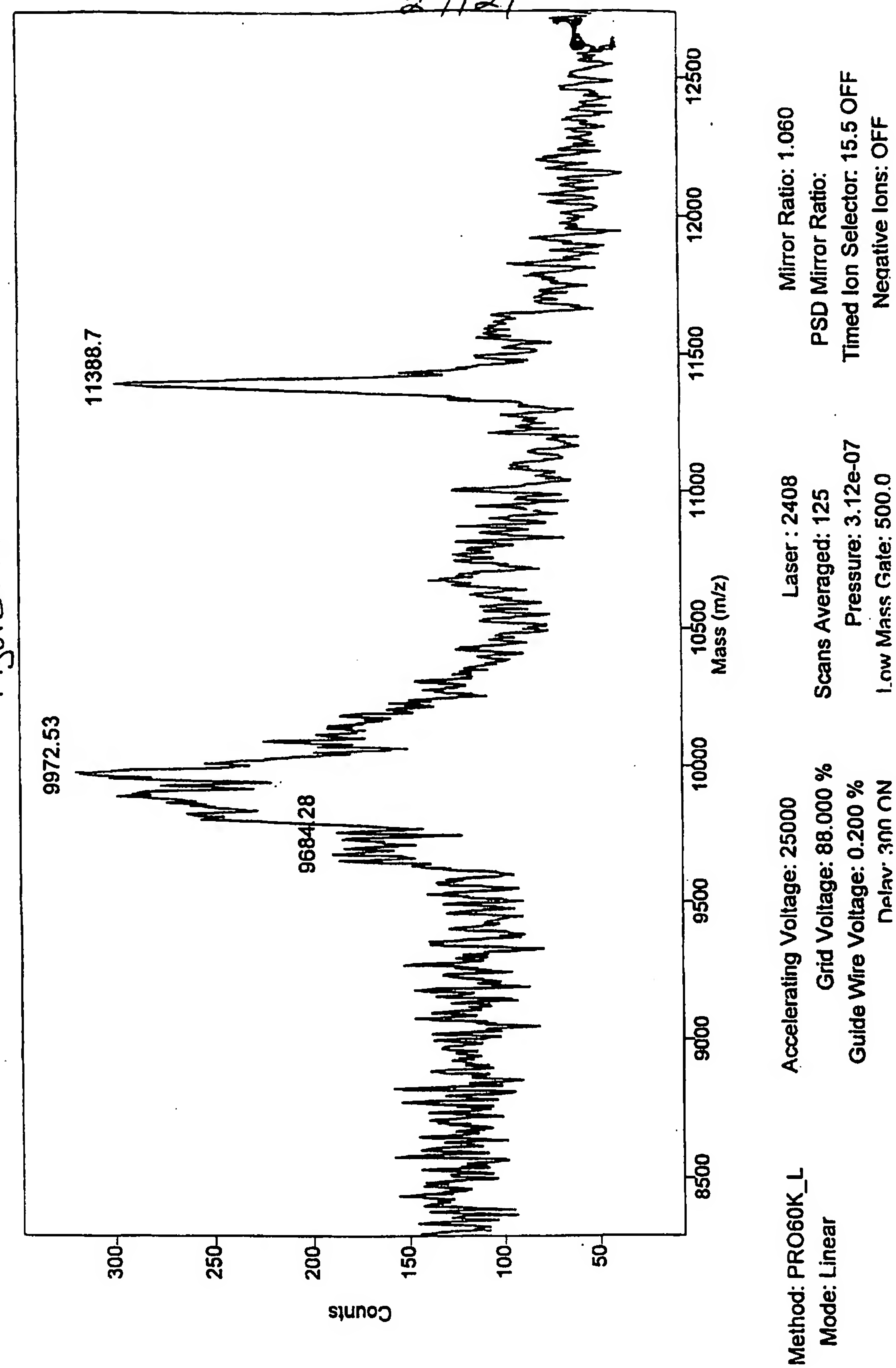
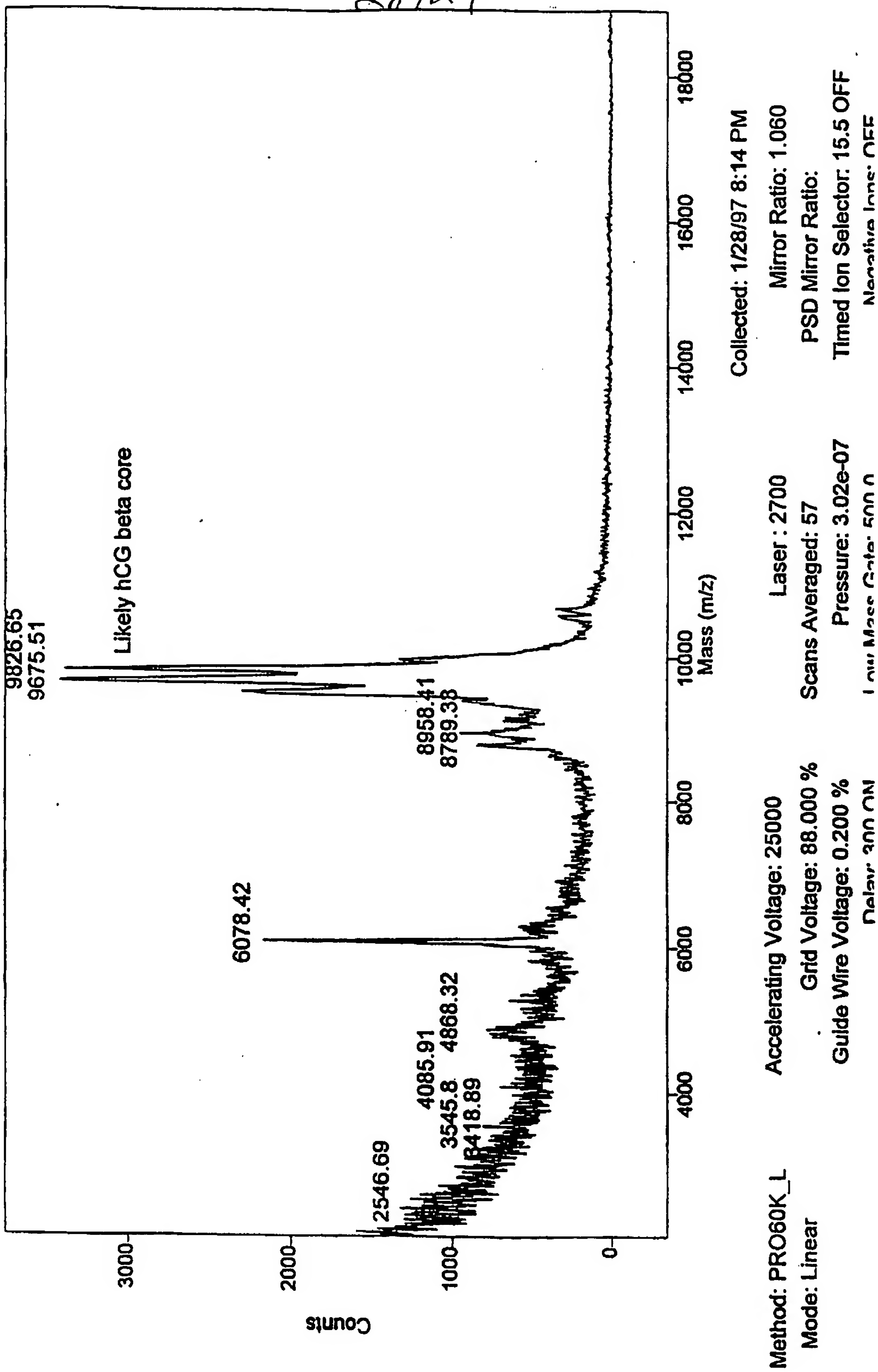


Figure 17C

Figure 17D



Likely hCG beta core
9826.65
9675.51
8958.41
8789.33
6078.42
4085.91
4868.32
3545.8
3418.89
2546.69
Counts
3000
2000
1000
0
4000
6000
8000
10000
12000
14000
16000
18000
Mass (m/z)
Method: PRO60K_L
Mode: Linear
Accelerating Voltage: 25000
Grid Voltage: 88.000 %
Guide Wire Voltage: 0.200 %
Laser : 2700
Scans Averaged: 57
Pressure: 3.02e-07
Collected: 1/28/97 8:14 PM
Mirror Ratio: 1.060
PSD Mirror Ratio:
Timed Ion Selector: 15.5 OFF

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Figure 17E